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(57) Abstract

The present invention provides CDR-grafted antibodies against human tissue factor that retain the high binding affinity of rodent monoclonal antibodies against tissue factor but have reduced immunogenicity. The present humanized antibodies are potent anticoagulants and are thus useful in the treatment and prophylaxis of human thrombotic disease. The invention also provides methods of making the CDR-grafted antibodies and pharmaceutical compositions for the attenuation or prevention of coagulation.

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CDR-GRAFTED ANTI-TISSUE FACTOR ANTIBODIES AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

Monoclonal antibodies capable of inhibiting tissue factor (TF) are useful as anticoagulants. Conventional rodent monoclonal antibodies, however, have limited use in human therapeutic and diagnostic applications due to immunogenicity and short serum half-life. The present invention provides CDR-grafted monoclonal antibodies against TF that retain the high binding affinity of rodent antibodies but have reduced immunogenicity. The present humanized antibodies are potent anticoagulants and are thus useful in the treatment and prophylaxis of human thrombotic disease. The invention also provides methods of making the CDR-grafted antibodies and pharmaceutical compositions for the attenuation or prevention of coagulation.

BACKGROUND OF THE INVENTION

The coagulation of blood involves a cascading series of reactions leading to the formation of fibrin. The coagulation cascade consists of two overlapping pathways, both of which are required for hemostasis. The intrinsic pathway comprises protein factors present in circulating blood, while the extrinsic pathway requires tissue factor, which is expressed on the cell surface of a variety of tissues in response to vascular injury. Davie et al., 1991, Biochemistry 30:10363. Agents that interfere with the coagulation cascade, such

as heparin and coumarin derivatives, have well-known therapeutic uses in the prophylaxis of venous thrombosis. Goodman and Gilman, eds., 1980, *The Pharmacological Basis of Therapeutics*, MacMillan Publishing Co., Inc., New York.

Tissue factor (TF) has been investigated as a target for anticoagulant therapy. TF is a membrane glycoprotein that functions as a receptor for factor VII and VIIa and thereby initiates the extrinsic pathway of the coagulation cascade in response to vascular injury. In addition to its role in the maintenance of hemostasis by initiation of blood clotting, TF has been implicated in pathogenic conditions. Specifically, the synthesis and cell surface expression of TF has been implicated in vascular disease (Wilcox *et al*., 1989, *Proc. Natl. Acad. Sci.* *86*:2839) and gram-negative septic shock (Warr *et al*., 1990, *Blood* *75*:1481).

Ruf *et al*. (1991, *Thrombosis and Haemostasis* *66*:529) characterized the anticoagulant potential of murine monoclonal antibodies against human TF. The inhibition of TF function by most of the monoclonal antibodies that were assessed was dependent upon the dissociation of the TF/VIIa complex that is rapidly formed when TF contacts plasma. Such antibodies were thus relatively slow inhibitors of TF in plasma. One monoclonal antibody, TF8-5G9, was capable of inhibiting the TF/VIIa complex without dissociation of the complex, thus providing an immediate anticoagulant effect in plasma. Ruf *et al*. suggest that mechanisms that inactivate the TF/VIIa complex, rather than prevent its formation, may provide strategies for interruption of coagulation *in vivo*.

The therapeutic use of monoclonal antibodies against TF is limited in that currently available monoclonals are of rodent origin. The use of rodent antibodies in human therapy presents numerous problems, the most significant of which is immunogenicity. Repeated doses of rodent monoclonal antibodies have been found to elicit an anti-immunoglobulin response termed human anti-mouse antibody (HAMA), which can result in immune complex disease and/or neutralization of the therapeutic antibody. See, e.g., Jaffers et al. (1986) Transplantation 41:572. While the use of human monoclonal antibodies would address this limitation, it has proven difficult to generate large amounts of human monoclonal antibodies by conventional hybridoma technology.

Recombinant technology has been used in an effort to construct "humanized" antibodies that maintain the high binding affinity of rodent monoclonal antibodies but exhibit reduced immunogenicity in humans. Chimeric antibodies have been produced in which the variable (V) region of a mouse antibody is combined with the constant (C) region of a human antibody in an effort to maintain the specificity and affinity of the rodent antibody but reduce the amount of protein that is non-human and thus immunogenic. While the immune response to chimeric antibodies is generally reduced relative to the corresponding rodent antibody, the immune response cannot be completely eliminated, because the mouse V region is capable of eliciting an immune response. Lobuglio et al. (1989) Proc. Natl. Acad. Sci. 86:4220; Jaffers et al. (1986) Transplantation 41:572.

In a recent approach to reducing immunogenicity of rodent antibodies, only the rodent complementarity determining regions (CDRs), rather than the entire V domain, are transplanted to a human antibody. Such humanized antibodies are known as CDR-grafted antibodies. CDRs are regions of hypervariability in the V regions that are flanked by relatively conserved regions known as framework (FR) regions. Each V domain contains three CDRs flanked by four FRs. The CDRs fold to form the antigen binding site of the antibody, while the FRs support the structural conformations of the V domains. Thus by transplanting the rodent CDRs to a human antibody, the antigen binding domain can theoretically also be transferred. Owens et al. (1994) J. Immunol. Methods 168:149 and Winter et al. (1993) Immunology Today 14:243 review the development of CDR-grafted antibodies.

Orlandi et al. (1989) Proc. Natl. Acad. Sci. USA 86:3833 constructed a humanized antibody against the relatively simple hapten nitrophenacetyl (NP). The CDR-grafted antibody contained mouse CDRs and human FRs, and exhibited NP binding activity similar to the native mouse antibody. However, the construction of CDR-grafted antibodies recognizing more complex antigens has resulted in antibodies having binding activity significantly lower than the native rodent antibodies. In numerous cases it has been demonstrated that the mere introduction of rodent CDRs into a human antibody background is insufficient to maintain full binding activity, perhaps due to distortion of the CDR conformation by the human FR.

For example, Gorman et al. (1991) Proc. Natl. Acad. Sci. 88:4181 compared two humanized antibodies against human CD4 and observed considerably different avidies depending upon the particular human framework region of the humanized antibody. Co et al. (1991) Proc. Natl. Acad. Sci. USA 88:2869 required a refined computer model of the murine antibody of interest in order to identify critical amino acids to be considered in the design of a humanized antibody. Kettleborough et al. (1991) Protein Engineering 4:773 report the influence of particular FR residues of a CDR-grafted antibody on antigen binding, and propose that the residues may directly interact with antigen, or may alter the conformation of the CDR loops. Similarly, Singer et al. (1993) J. Immunol. 150:2844 report that optimal humanization of an anti-CD18 murine monoclonal antibody is dependent upon the ability of the selected FR to support the CDR in the appropriate antigen binding conformation. Accordingly, recreation of the antigen-binding site requires consideration of the potential intrachain interactions between the FR and CDR, and manipulation of amino acid residues of the FR that maintain contacts with the loops formed by the CDRs. While general theoretical guidelines have been proposed for the design of humanized antibodies (see, e.g., Owens et al.), in all cases the procedure must be tailored and optimized for the particular rodent antibody of interest.

There is a need in the art for humanized antibodies with reduced immunogenicity and comparable binding affinity relative to the parent rodent antibody for various therapeutic applications. In particular,

there is a need for a humanized antibody against human tissue factor having anticoagulant activity and useful in the treatment and prevention of thrombotic disease.

SUMMARY OF THE INVENTION

The present invention is directed to CDR-grafted antibodies capable of inhibiting human tissue factor wherein the CDRs are derived from a non-human monoclonal antibody against tissue factor and the FR and constant (C) regions are derived from one or more human antibodies. In a preferred embodiment, the murine monoclonal antibody is TF8-5G9.

In another embodiment, the present invention provides a method of producing a CDR-grafted antibody capable of inhibiting human tissue factor which method comprises constructing one or more expression vectors containing nucleic acids encoding CDR-grafted antibody heavy and light chains, transfecting suitable host cells with the expression vector or vectors, culturing the transfected host cells, and recovering the CDR-grafted antibody.

The present invention also provides a method of attenuation of coagulation comprising administering a CDR-grafted antibody capable of inhibiting human tissue factor to a patient in need of such attenuation.

The present invention further provides a method of treatment or prevention of thrombotic disease comprising administering a CDR-grafted antibody capable of inhibiting human tissue factor to a patient in need of such treatment or prevention. In a preferred

embodiment, the thrombotic disease is intravascular coagulation, arterial restenosis or arteriosclerosis.

Another embodiment of the present invention is directed to a pharmaceutical composition comprising CDR-grafted antibodies capable of inhibiting human tissue factor and further comprising a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 provides the nucleotide and deduced amino acid sequences of the heavy chain of murine monoclonal antibody TF8-5G9.

Fig. 2 provides the nucleotide and deduced amino acid sequences of the light chain of murine monoclonal antibody TF8-5G9.

Fig. 3 is a graph depicting the ability of CDR-grafted antibody TF8HCDR1 x TF8LCDR1 to bind to human tissue factor and to compete with murine monoclonal antibody TF85G9 for binding to tissue factor. Solid symbols indicate direct binding of TF8HCDR1 x TF8LCDR1 and the positive control chimeric TF85G9 to tissue factor. Open symbols indicate competition binding of TF8HCDR1 x TF8LCDR1 or chimeric TF85G9 with murine monoclonal antibody TF85G9.

Fig. 4 presents the DNA sequence of expression vector pEe6TF8HCDR20 and the amino acid sequence of the coding regions of the CDR-grafted heavy chain TF8HCDR20.

Fig. 5 presents the DNA sequence of expression vector pEe12TF8LCDR3 and the amino acid sequence of the coding regions of the CDR-grafted light chain TF8LCDR3.

Fig. 6 is a graph depicting the ability of CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to bind to human tissue factor.

Fig. 7 is a graph depicting the ability of CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to compete with murine monoclonal antibody TF85G9 for binding to tissue factor.

Fig. 8 is a graph depicting the ability of CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to inhibit factor X activation.

Fig. 9 provides expression vector pEe6TF8HCDR20 resulting from the subcloning of CDR-grafted heavy chain TF8HCDR20 into myeloma expression vector pEehCMV-BglI. The following abbreviations are used: VH is the CDR-grafted heavy chain variable region; Cγ4 is the human IgG4 constant region; pA is the polyadenylation signal; ampR is the ß-lactamase gene; and hCMV is human cytomegalovirus.

Fig. 10 provides expression vector pEe12TF8LCDR3 resulting from the subcloning of CDR-grafted light chain TF8LCDR3 into myeloma expression vector pEe12. The following abbreviations are used: VL is the CDR-grafted light chain variable region; CK is the human kappa constant region; SVE is the SV40 early promoter; GS is glutamine synthetase cDNA. Other abbreviations are as noted in Fig. 9.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides CDR-grafted antibodies capable of inhibiting human tissue factor wherein the CDRs are derived from a non-human monoclonal

antibody against tissue factor and the FR and C regions are derived from one or more human antibodies. The present invention further provides methods of making and using the subject CDR-grafted antibodies.

In accordance with the present invention, the CDR-grafted antibody is an antibody in which the CDRs are derived from a non-human antibody capable of binding to and inhibiting the function of human tissue factor, and the FR and C regions of the antibody are derived from one or more human antibodies. The CDRs derived from the non-human antibody preferably have from about 90% to about 100% identity with the CDRs of the non-human antibody, although any and all modifications, including substitutions, insertions and deletions, are contemplated so long as the CDR-grafted antibody maintains the ability to bind to and inhibit tissue factor. The regions of the CDR-grafted antibodies that are derived from human antibodies need not have 100% identity with the human antibodies. In a preferred embodiment, as many of the human amino acid residues as possible are retained in order than immunogenicity is negligible, but the human residues, in particular residues of the FR region, are substituted as required and as taught hereinbelow in accordance with the present invention. Such modifications as disclosed herein are necessary to support the antigen binding site formed by the CDRs while simultaneously maximizing the humanization of the antibody.

Non-human monoclonal antibodies against human tissue factor from which the CDRs can be derived are known in the art (Ruf et al., 1991; Morrisey et al., 1988, Thrombosis Research 52:247) or can be produced by

well-known methods of monoclonal antibody production (see, e.g. Harlow et al., eds., 1988, Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratories, Cold Spring Harbor, New York). Purified human tissue factor against which monoclonal antibodies can be raised is similarly well-known (Morrisey et al., 1987, Cell 50:129) and available to the skilled artisan. Murine monoclonal antibodies, and in particular murine monoclonal antibody TF8-5G9 disclosed by Ruf et al. and Morrisey et al., 1988, Thrombosis Research 52:247, and U.S. Patent No. 5,223,427 are particularly preferred.

The ordinarily skilled artisan can determine the sequences of the CDRs by reference to published scientific literature or sequence databanks, or by cloning and sequencing the heavy and light chains of the antibodies by conventional methodology. In accordance with the present invention, the cDNA and amino acid sequences of the heavy chain (SEQ ID NOS:1 and 2, respectively) and light chain (SEQ ID NOS:3 and 4, respectively) of murine monoclonal antibody TF8-5G9 are provided. The cDNA and deduced amino acid sequence of the murine TF8-5G9 heavy chain is provided at Figure 1. The cDNA and deduced amino acid sequence of the murine TF8-5G9 light chain is provided at Figure 2.

Each of the heavy and light chain variable regions contain three CDRs that combine to form the antigen binding site. The three CDRs are surrounded by four FR regions that primarily function to support the CDRs. The sequences of the CDRs within the sequences of the variable regions of the heavy and light chains can be identified by computer-assisted alignment according to Kabat et al. (1987) in Sequences of Proteins of

Immunological Interest, 4th ed., United States Department of Health and Human Services, US Government Printing Office, Washington, D.C., or by molecular modeling of the variable regions, for example utilizing the ENCAD program as described by Levitt (1983) J. Mol. Biol. 168:595.

In a preferred embodiment the CDRs are derived from murine monoclonal antibody TF8-5G9. The preferred heavy chain CDRs have the following sequences:

CDR1	DDYMH	(SEQ ID NO:5)
CDR2	LIDPENGNTIYDPKFQG	(SEQ ID NO:6)
CDR3	DNSYYFDY	(SEQ ID NO:7)

The preferred light chain CDRs have the following sequences:

CDR1	KASQDIRKYLN	(SEQ ID NO:8)
CDR2	YATSLAD	(SEQ ID NO:9)
CDR3	LQHGESPYT	(SEQ ID NO:10)

The sequences of the CDRs of the murine or other non-human antibody, and in particular the sequences of the CDRs of TF8-5G9, may be modified by insertions, substitutions and deletions to the extent that the CDR-grafted antibody maintains the ability to bind to and inhibit human tissue factor. The ordinarily skilled artisan can ascertain the maintenance of this activity by performing the functional assays described hereinbelow. The CDRs can have, for example, from about 50% to about 100% homology to the CDRs of SEQ ID NOS:5-10. In a preferred embodiment the CDRs have from about

80% to about 100% homology to the CDRs of SEQ ID NOS:5-10. In a more preferred embodiment the CDRs have from about 90% to about 100% homology to the CDRs of SEQ ID NOS:5-10. In a most preferred embodiment the CDRs have from about 100% homology to the CDRs of SEQ ID NOS:5-10.

The FR and C regions of the CDR-grafted antibodies of the present invention are derived from one or more human antibodies. Human antibodies of the same class and type as the antibody from which the CDRs are derived are preferred. The FR of the variable region of the heavy chain is preferably derived from the human antibody KOL (Schmidt et al., 1983, Hoppe-Seyler's Z. Physiol. Chem. 364:713) The FR of the variable region of the light chain is preferably derived from the human antibody REI (Epp et al., 1974, Eur. J. Biochem. 45:513). In accordance with the present invention, it has been discovered that certain residues of the human FR are preferably replaced by the corresponding residue of the non-human antibody from which the CDRs are derived. For example, certain FR residues of TF8-5G9 are preferably retained to achieve optimal binding to antigen.

For convenience, the numbering scheme of Kabat et al. has been adopted herein. Residues are designated by lower case numbers or hyphens as necessary to conform the present sequences to the standard Kabat numbered sequence.

In accordance with the present invention, residues that are retained in the FR region, i.e residues that are not replaced by human FR residues, are determined according to the following guidelines. Residues that are idiosyncratic to the parent antibody,

e.g. TF8-5G9, relative to a human consensus sequence of Kabat et al, are retained. Residues of the parent antibody that are in agreement with the consensus sequence are retained if the corresponding residue of the human antibody, e.g. KOL or REI, is idiosyncratic. Residues that are part of the antibody loop canonical structures defined by Chothia et al. (1989) Nature 342:877, such as residue 71 of the heavy and light chains, are retained. FR residues predicted to form loops, such as residues 28-30 of the heavy chain, are retained. FR residues predicted to influence the conformation of the CDRs such as residues 48 and 49 preceding CDR2 of the heavy chain, are retained. Residues that have been demonstrated to be critical in the humanization of other antibodies may also be retained. The foregoing guidelines are followed to the extent necessary to support the antigen binding site formed by the CDRs while simultaneously maximizing the humanization of the antibody.

The amino acid sequence of a representative CDR-grafted heavy chain variable region derived from murine monoclonal antibody TF8-5G9 and human antibody KOL is shown below. The CDR-grafted heavy chain is designated TF8HCDR1; murine residues were retained in the FR at residues 6, 17, 23, 24, 28, 29, 30, 48, 49, 68, 71, 73, 78, 88 and 91. CDRs are underlined.

```
          10         20         30    35ab          50
QVQLVQSGGG VVQPGRLLRL SCKASGFNIK DYYMH--WVR QAPGKGLEWIG
52abc      60         70            80 82abc      90
LIDP--ENGNTIYD PKFQGRFSIS ADTSK--NTAFL QMDSLRPEDTAVY
100        110
YCARDNSYYF DYWGQGTPVT VSS (SEQ ID NO:11)
```

The amino acid sequence of a representative CDR-grafted light chain variable region derived from murine monoclonal antibody TF8-5G9 and human antibody REI is shown below. The CDR-grafted light chain is designated TF8LCDR1; murine residues were retained in the FR at residues 39, 41, 46 and 105. CDRs are underlined.

```
          10         20         30         40         50
DIQMTQSPSS LSASVGDRVT ITCKASQDIR KYLNWYQQK WKAPKTLIYY
          60         70         80         90        100
ATSLADGVPS RFSGSGSGTD YTFTISSLQP EDIATYYCLQ HGESPYTFGQ

GTKLEITR  (SEQ ID NO:12)
```

A CDR-grafted antibody containing variable regions TF8HCDR1 and TF8LCDR1 has been demonstrated in accordance with the present invention to be as effective as murine monoclonal antibody TF8-5G9 in binding to human tissue factor. It has been further discovered in accordance with the present invention, by examination of the molecular structure of murine monoclonal antibody TF8-5G9, and by design, construction, and analysis of CDR-grafted antibodies, that the FR regions can be further humanized without the loss of antigen binding activity. In particular, the FR region may retain the human FR residue at residues 6, 17, 68, 73 and 78 of the heavy chain, and residues 39, 41, 16 and 105 of the light chain, with maintenance of antigen binding activity.

In a most preferred embodiment, the heavy chain variable region contains a FR derived from human antibody KOL in which murine monoclonal antibody TF8-5G9

residues are retained at amino acids 23, 24, 28, 29, 30, 48, 49, 71, 88 and 91. The preferred heavy chain variable region is designated TF8HCDR20 and has the following sequence.

```
        10        20        30   35ab            50
QVQLVESGGG VVQPGRSLRL SCKASGFNIK DYYMH--WVR QAPGKGLEWIGL

52abc      60        70        80 82abc     90       100
IDP--ENGNTIYD PKFQGRFTIS ADNSKNTLFL QMDSLRPEDTAVY YCARDNSYYF

        110
DYWGQGTPVT VSS  (SEQ ID NO:13)
```

In a most preferred embodiment, the light chain variable region contains a FR derived from human antibody REI in which murine monoclonal antibody TF8-5G9 residues are retained at amino acids 39 and 105. The preferred light chain variable region is designated TF8LCDR20 and has the following sequence.

```
         10         20         30         40         50
DIQMTQSPSS LSASVGDRVT ITCKASQDIR KYLNWYQQKP GKAPKLLIYY
         60         70         80         90        100
ATSLADGVPS RFSGSGSGTD YTFTISSLQP EDIATYYCLQ HGESPYTFGQ
GTKLEITR  (SEQ ID NO:14)
```

It is within the ken of the ordinarily skilled artisan to make minor modifications of the foregoing sequences, including amino acid substitutions, deletions and insertions. Any such modifications are within the scope of the present invention so long as the resulting CDR-grafted antibody maintains the ability to bind to and inhibit human tissue factor. The ordinarily skilled artisan can assess the activity of the CDR-grafted

antibody with reference to the functional assays described hereinbelow.

The human constant region of the CDR-grafted antibodies of the present invention is selected to minimize effector function. The intended use of the CDR-grafted antibodies of the present invention is to block the coagulation cascade by inhibition of tissue factor, and thus antibody effector functions such as fixation of complement are not desirable. Antibodies with minimal effector functions include IgG2, IgG4, IgA, IgD and IgE. In a preferred embodiment of the present invention, the heavy chain constant region is the human IgG4 constant region, and the light chain constant region is the human IgG4 kappa constant region.

In that effector functions may not be desirable for therapeutic uses, the present invention further contemplates active fragments of the CDR-grafted antibodies, and in particular Fab fragments and $F(ab')_2$ fragments. Active fragments are those fragments capable of inhibiting human tissue factor. Fab fragments and $F(ab')_2$ fragments may be obtained by conventional means, for example by cleavage of the CDR-grafted antibodies of the invention with an appropriate proteolytic enzyme such as papain or pepsin, or by recombinant production. The active fragments maintain the antigen binding sites of the CDR-grafted antibodies and thus are similarly useful therapeutically.

The ability of the CDR-grafted antibodies designed and constructed as taught in accordance with the present invention to bind and inhibit human tissue factor can be assessed by functional assays. For example, in a rapid and convenient assay, expression

vectors containing nucleic acids encoding the CDR-grafted heavy and light chains can be co-transfected into suitable host cells and transiently expressed. The resulting antibodies can be assessed by standard assays for ability to bind human tissue factor, and for ability to compete for binding to tissue factor with the non-human antibody from which the CDRs are derived.

For example, transient expression of nucleic acids encoding the CDR-grafted heavy and light chains in COS cells provides a rapid and convenient system to test antibody gene expression and function. Nucleic acids encoding the CDR-grafted heavy and light chains, respectively, are cloned into a mammalian cell expression vector, for example pSG5, described by Green et al. (1988) Nucleic Acids Res. 16:369 and commercially available from Stratagene Cloning Systems, La Jolla, CA. The pSG5 expression vector provides unique restriction sites for the insertion of the heavy and light chain genes, and in vivo expression is under the control of the SV40 early promoter. Transcriptional termination is signaled by the SV40 polyadenylation signal sequence.

The pSG5-based expression vectors containing nucleic acids encoding the heavy and light chains are cotransfected into COS cells and cultured under conditions suitable for transient expression. Cell culture media is then harvested and examined for antibody expression, for example by an enzyme linked immunosorbent assay (ELISA), to determine that suitable levels of antibody have been produced. An ELISA may then be used to assess the ability of the CDR-grafted antibody to bind to human tissue factor. Human tissue factor is immobilized on a microtiter plate and the COS

cell supernatant containing the CDR-grafted antibody is added followed by an incubation at room temperature for about one hour. The plates are then washed with a suitable detergent-containing buffer such as phosphate buffered saline (PBS)/Tween, followed by the addition of the components of a suitable detection system. For example, horseradish peroxidase conjugated goat anti-human kappa chain polyclonal antibody is added, followed by washing, followed by addition of substrate for horseradish peroxidase, and detection. The CDR-grafted antibodies within the scope of the present invention are those which are capable of binding to human tissue factor to a degree comparable to the non-human antibody from which the CDRs are derived as determined by the foregoing assay.

The ability of the CDR-grafted antibodies to inhibit the activity of human tissue factor <u>in vivo</u> can be conveniently assessed by the following <u>in vitro</u> assay that mimics <u>in vivo</u> coagulation events. In response to vascular injury <u>in vivo</u>, tissue factor binds to factor VII and facilitates the conversion of factor VII to a serine protease (factor VIIa). The factor VIIa-tissue factor complex converts factor X to a serine protease (factor Xa). Factor Xa forms a complex with factor Va (from the intrinsic coagulation pathway), resulting in the conversion of prothrombin to thrombin, which in turn results in the conversion of fibrinogen to fibrin. In a convenient <u>in vitro</u> functional assay, tissue factor is incubated in the presence of factor VIIa and the CDR-grafted anti-tissue factor antibody produced in the transient expression system described above. Factor X is added and the reaction mixture is incubated, followed

by an assay for factor Xa activity utilizing a chromogenic substrate for factor Xa (Spectrozyme FXa, American Diagnostica, Inc., Greenwich, CT). The ability of the CDR-grafted antibody to inhibit factor X activation thus provides a measure of the ability of the CDR-grafted antibody to inhibit the activity of human tissue factor.

The CDR-grafted antibodies within the scope of the present invention are those which are capable of inhibiting human tissue factor to a degree comparable to the non-human antibody from which the CDRs are derived as determined by the foregoing assay. In one embodiment, the CDR-grafted antibody has at least 50% of the inhibitory activity of TF8-5G9 for human tissue factor. In a preferred embodiment, the CDR-grafted antibody has at least 70% of the inhibitory activity of TF8-5G9 for human tissue factor. In a more preferred embodiment, the CDR-grafted antibody has at least 80% of the inhibitory activity of TF8-5G9 for human tissue factor. In a most preferred embodiment, the CDR-grafted antibody has at least 90% of the inhibitory activity of TF8-5G9 for human tissue factor.

In another embodiment, the present invention provides a method of producing a CDR-grafted antibody capable of inhibiting human tissue factor. The method comprises constructing an expression vector containing a nucleic acid encoding the CDR-grafted antibody heavy chain and an expression vector containing a nucleic acid encoding the CDR-grafted antibody light chain, transfecting suitable host cells with the expression vectors, culturing the transfected host cells under conditions suitable for the expression of the heavy and

light chains, and recovering the CDR-grafted antibody. Alternately, one expression vector containing nucleic acids encoding the heavy and light chains may be utilized.

Standard molecular biological techniques, for example as disclosed by Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual Cold Spring Harbor Press, Cold Spring Harbor, NY may be used to obtain nucleic acids encoding the heavy and light chains of the CDR-grafted antibodies of the present invention. A nucleic acid encoding the CDR-grafted variable domain may be constructed by isolating cDNA encoding the antibody to be humanized, e.g. murine monoclonal antibody TF8-5G9, by conventional cloning methodology from the hybridoma producing the antibody, or by polymerase chain reaction (PCR) amplification of the variable region genes, as described for example by Winter et al., followed by site-directed mutagenesis to substitute nucleotides encoding the desired human residues into the FR regions. Alternately, the cDNA encoding the human antibody can be isolated, followed by site-directed mutagenesis to substitute nucleotides encoding the desired murine residues into the CDRs.

Nucleic acids encoding the CDR-grafted variable domain may also be synthesized by assembling synthetic oligonucleotides, for example utilizing DNA polymerase and DNA ligase. The resulting synthetic variable regions may then be amplified by PCR. Nucleic acids encoding CDR-grafted variable domains may also be constructed by PCR strand overlap methods that are known in the art and reviewed by Owens et al.

Accordingly, having determined the desired amino acid sequences of the CDR-grafted variable domains in accordance with the present invention, the ordinarily skilled artisan can obtain nucleic acids encoding the variable domains. Further, the skilled artisan is aware that due to the degeneracy of the genetic code, various nucleic acid sequences can be constructed that encode the CDR-grafted variable domains. All such nucleic acid sequence are contemplated by the present invention.

The nucleic acids encoding the CDR-grafted variable domains are linked to appropriate nucleic acids encoding the human antibody heavy or light chain constant region. Nucleic acid sequences encoding human heavy and light chain constant regions are known in the art. It is within the ken of the ordinarily skilled artisan to include sequences that facilitate transcription, translation and secretion, for example start codons, leader sequences, the Kozak consensus sequence (Kozak, 1987, J. Mol. Biol. 196:947) and the like, as well as restriction endonuclease sites to facilitate cloning into expression vectors.

The present invention thus further provides nucleic acids encoding the heavy and light chains of CDR-grafted antibodies capable of inhibiting human tissue factor wherein the CDRs are derived from a murine monoclonal antibody against tissue factor and the FR and C regions are derived from one or more human antibodies.

In accordance with the present invention, representative nucleic acids encoding CDR-grafted heavy and light chains were constructed. The CDR-grafted heavy chain comprises a variable region containing FR regions derived from human antibody KOL and CDRs derived

from murine monoclonal antibody TF8-5G9 and further comprises a constant region derived from the heavy chain of human IgG4. The CDR-grafted light chain comprises a variable region containing FR regions derived from human antibody REI and CDRs derived from murine monoclonal antibody TF8-5G9 and further comprises a constant region derived from human IgG4 kappa chain. Nucleic acids encoding the heavy and light chains were constructed by assembling the variable regions from synthetic nucleotides, amplifying the assembled variable regions by PCR, purifying the amplified nucleic acids, and ligating the nucleic acid encoding the variable region into a vector containing a nucleic acid encoding the appropriate human constant region.

The sequences of representative nucleic acids encoding CDR-grafted heavy and light chains are presented as nucleotides 1-2360 of SEQ ID NO:15 and nucleotides 1-759 of SEQ ID NO:20, respectively.

The nucleic acid sequence encoding a preferred heavy chain (nucleotides 1-2360 of SEQ ID NO:15) is designated the TF8HCDR20 gene. The nucleic acid sequence contains the following regions: 5' EcoRI restriction site (nucleotides 1-6); Kozak sequence (nucleotides 7-15); start codon and leader sequence (nucleotides 16-72); CDR-grafted variable region (nucleotides 73-423); human IgG4 CH1 domain (nucleotides 424-717); human IgG4 intron 2 (nucleotides 718-1110); human IgG4 hinge (nucleotides 1111-1146); human IgG4 intron 3 (nucleotides 1147-1267); human IgG4 CH2 domain (nucleotides 1268-1594); human IgG4 intron 4 (nucleotides 1595-1691); human IgG4 CH3 domain (nucleotides 1692-2012); 3' untranslated region

(nucleotides 2013-2354); 3' BamHI end spliced to BclI site of expression vector (nucleotides 2355-2360).

The nucleic acid sequence encoding a preferred light chain gene (nucleotides 1-759 of SEQ ID NO:20) is designated the TF8LCDR3 gene. The nucleic acid sequence contains the following regions: 5' EcoRI restriction site (nucleotides 1-5); Kozak sequence (nucleotides 6-8); start codon and leader sequence (nucleotides 9-68); CDR-grafted variable region (nucleotides 69-392); human kappa constant region (nucleotides 393-710); 3' untranslated region (nucleotides 711-753); 3' BamHI end spliced to BclI site of expression vector (nucleotides 754-759).

The foregoing preferred sequences can be modified by the ordinarily skilled artisan to take into account degeneracy of the genetic code, and to make additions, deletions, and conservative and nonconservative substitutions that result in a maintenance of the function of the nucleic acid, i.e. that it encodes a heavy or light chain of a CDR-grafted antibody capable of inhibiting human tissue factor. Restriction sites and sequences that facilitate transcription and translation may be altered or substituted as necessary depending upon the vector and host system chosen for expression.

Suitable expression vectors and hosts for production of the CDR-grafted antibodies of the present invention are known to the ordinarily skilled artisan. The expression vectors contain regulatory sequences, such as replicons and promoters, capable of directing replication and expression of heterologous nucleic acids sequences in a particular host cell. The vectors may

also contain selection genes, enhancers, signal sequences, ribosome binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and so on. The vectors may be constructed by conventional methods well-known in the art, or obtained from commercial sources. The expression vectors preferably have convenient restriction sites at which the nucleic acids encoding the antibody chains of the invention are inserted. Myeloma expression vectors in which antibody gene expression is driven by the human cytomegalovirus promoter-enhancer or are particularly preferred.

Expression vectors containing a nucleic acid encoding the CDR-grafted heavy chain under the control of a suitable promoter and expression vectors containing a nucleic acid encoding the CDR-grafted light chain under the control of a suitable promoter are cotransfected into a suitable host cell. In another embodiment, nucleic acids encoding both heavy and light chains are provided in a single vector for transfection of a suitable host cell.

Suitable host cells or cell lines for expression of the CDR-grafted antibodies of the present invention include bacterial cells, yeast cells, insect cells, and mammalian cells such as Chinese hamster ovary (CHO) cells, COS cells, fibroblast cells and myeloid cells. Mammalian cells are preferred. CHO, COS and myeloma cells are particularly preferred. Myeloma cells are preferred for establishing permanent CDR-grafted antibody producing cell lines. Expression of antibodies in myeloma cells, bacteria, and yeast is reviewed by

Sandhu (1992) Critical Reviews in Biotechnology 12:437. Expression in mammalian cells is reviewed by Owen et al..

Transfection of host cells by the expression vectors containing nucleic acids encoding the CDR-grafted heavy and light chains can be accomplished by methods well-known to one of ordinary skill in the art. Such methods include, for example, calcium chloride transfection, calcium phosphate transfection, lipofection and electroporation. Suitable culture methods and conditions for the production of the CDR-grafted antibodies are likewise well-known in the art. The CDR-grafted antibodies can be purified by conventional methods, including ammonium sulfate precipitation, affinity chromatography, gel electrophoresis, and the like. The ability of the CDR-grafted antibodies to bind to and inhibit human tissue factor can be assessed by the in vitro assays described above.

The CDR-grafted antibodies of the present invention have a variety of utilities. For example, the antibodies are capable of binding to human tissue factor and thus are useful in assays for human tissue factor from body fluid samples, purification of human tissue factor, and so on.

The CDR-grafted antibodies of the present invention are capable of inhibiting human tissue factor. Human tissue factor is well-known to be an essential element in the human coagulation cascade. The ability of the antibodies of the present invention to disrupt the coagulation cascade is demonstrated by in vitro assays in which the antibodies prevent factor X activation. Accordingly, the present antibodies are

useful in the attenuation of coagulation. The present invention thus provides a method of attenuation of coagulation comprising administering a therapeutically effective amount of CDR-grafted antibody capable of inhibiting human tissue factor to a patient in need of such attenuation.

Numerous thrombotic disorders are characterized by excessive or inappropriate coagulation and are effectively treated or prevented by administration of agents that interfere with the coagulation cascade. Accordingly, the present invention further provides a method of treatment or prevention of a thrombotic disorder comprising administering a therapeutically effective amount of a CDR-grafted antibody capable of inhibiting human tissue factor to a patient in need of such treatment or prevention. In a preferred embodiment, the thrombotic disorder is intravascular coagulation, arterial restenosis or arteriosclerosis. The antibodies of the invention may be used in combination with other antibodies or therapeutic agents.

A therapeutically effective amount of the antibodies of the present invention can be determined by the ordinarily skilled artisan with regard to the patient's condition, the condition being treated, the method of administration, and so on. A therapeutically effective amount is the dosage necessary to alleviate, eliminate, or prevent the thrombotic disorder as assessed by conventional parameters. For example, a therapeutically effective dose of a CDR-grafted antibody of the present invention may be from about 0.1 mg to about 20 mg per 70 kg of body weight. A preferred

dosage is about 1.0 mg to about 5 mg per 70 kg of body weight.

A patient in need of such treatment is a patient suffering from a disorder characterized by inappropriate or excessive coagulation, or a patient at risk of such a disorder. For example, anticoagulant therapy is useful to prevent postoperative venous thrombosis, and arterial restenosis following balloon angioplasty.

The CDR-grafted antibodies of the present invention are useful in the same manner as comparable therapeutic agents, and the dosage level is of the same order of magnitude as is generally employed with those comparable therapeutic agents. The present antibodies may be administered in combination with a pharmaceutically acceptable carrier by methods known to one of ordinary skill in the art.

Another embodiment of the present invention is directed to a pharmaceutical composition comprising a least one CDR-grafted antibody capable of inhibiting human tissue factor and further comprising a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well-known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The antibodies can be administered by well-known routes including oral and parenteral, e.g., intravenous, intramuscular, intranasal, intradermal, subcutaneous, and the like. Parenteral administration and particularly intravenous administration is preferred. Depending on the route of administration, the pharmaceutical composition may require protective coatings.

The pharmaceutical forms suitable for injectionable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the ultimate solution form must be sterile and fluid. Typical carriers include a solvent or dispersion medium containing, for example, water buffered aqueous solutions (i.e., biocompatible buffers), ethanol, polyol such as glycerol, propylene glycol, polyethylene glycol, suitable mixtures thereof, surfactants or vegetable oils. The antibodies may be incorporated into liposomes for parenteral administration. Sterilization can be accomplished by an art-recognized techniques, including but not limited to, addition of antibacterial or antifungal agents, for example, paraben, chlorobutanol, phenol, sorbic acid or thimersal. Further, isotonic agents such as sugars or sodium chloride may be incorporated in the subject compositions.

Production of sterile injectable solutions containing the subject antibodies is accomplished by incorporating these antibodies in the required amount in the appropriate solvent with various ingredients enumerated above, as required, followed by

sterilization, preferably filter sterilization. To obtain a sterile powder, the above solutions are vacuum-dried or freeze-dried as necessary.

The following examples further illustrate the present invention.

EXAMPLE 1

Isolation and Sequencing of TF8-5G9 Light Chain (LC) and Heavy Chain (HC)

Two DNA libraries were generated from oligo (dT)-primed TF8-5G9 hybridoma RNA utilizing standard molecular biology procedures as described by Sambrook et al. The cDNA was cloned into the Librarian II plasmid vector from Invitrogen (San Diego, CA), and the libraries were screened for cDNA clones encoding murine IgG HC and LC. A full-length cDNA clone for the heavy chain could not be isolated, despite the construction of two independent libraries. A random primed TF8-5G9 cDNA library was generated to obtain the missing 5' sequence of the heavy chain. Consequently, the heavy chain cDNA was in two pieces: a 5' clone of 390 nucleotides and a 3' clone of 1392 nucleotides. The two HC clones overlap by 292 nucleotides.

The HC and LC clones were completely sequenced by the dideoxy chain termination method of Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74:5463. To verify the variable region sequence, sequence was obtained from PCR-amplified cDNA that had been synthesized from total TF8-5G9 hybridoma RNA. Total TF8-5G9 hybridoma RNA was isolated by the guanidinium thiocyanate method of Chrigwin et al. (1970) Biochemistry 18:5294. cDNA was synthesized using the Perkin Elmer (Norwalk, CT) GeneAmp RNA Polymerase Chain Reaction (PCR) kit with an oligo (dT) primer. Components of the same kit were used in the PCR to amplify the LC and HC variable regions using primers based on the sequence that had been obtained for the cDNA clones. The amplified variable region

fragments were gel-purified and sequenced according to the method of Tracy <u>et al</u>. (1991) <u>BioTechniques</u> <u>11</u>:68 on a Model 373A Applied Biosystems, Inc. (Foster City, CA) automated fluorescent DNA sequencer. The sequence for TF8-5G9 LC and HC obtained from RNA amplification and the sequence obtained from the cDNA clones agreed. The TF8-5G9 HC variable region sequence with protein translation is shown in Figure 1 and SEQ ID NO:1, and that for the LC is shown in Figure 2 and SEQ ID NO:3.

EXAMPLE 2

Chimeric LC and HC Expression Vector Construction

In order to test the binding activity of the CDR-grafted anti-TF LC and HC individually, mouse-human chimeric TF8-5G9 LC and HC were constructed. This allowed the CDR-grafted LC to be tested for TF binding ability in combination with the chimeric HC, and the CDR-grafted HC to be tested in combination with the chimeric LC.

Primers were designed to amplify the TF8-5G9 LC variable region using as template cDNA clones in the Librarian II vector. The 5' primer was designed with an EcoRI site while the 3' primer was designed with a NarI site. PCR was used to amplify the LC variable region, generating a 433 bp fragment with a 5'EcoRI end and 3'NarI end. The fragment included the signal sequence from the TF8-5G9 LC cDNA clone but incorporated a 2 base change in the arginine codon immediately following the ATG start codon. This change retained the arginine residue but made the sequence conform to the Kozak consensus sequence in order to potentially improve translation of the LC mRNA. The PCR amplified LC variable region fragment was digested with EcoRI and NarI restriction enzymes and purified by electrophoresis on a 2% Nusieve, 1% Seakem agarose gel (FMC Bio Products, Rockland, ME).

The DNA was extracted from the gel slice and purified by the Geneclean (Bio 101, La Jolla, CA) procedure. The full-length chimeric TF8-5G9 LC gene was generated by cloning this DNA into the EcoRI and NarI sites of a pSP73 vector (Promega, Madison, WI) which

contains the human kappa constant region. The gene was isolated from the pSP73 vector by EcoRI digestion and subcloned into the EcoRI site of the pSG5 mammalian cell expression vector (Stratagene Cloning Systems, La Jolla, CA).

The chimeric TF8-5G9 HC gene was assembled in a manner similar to that of the chimeric LC. Since there was no full-length HC cDNA isolated from the Librarian II vector cDNA libraries, the HC variable region fragment that was generated by the PCR from total TF8-5G9 hybridoma cell RNA was used as the template. Primers which incorporated an EcoRI site at the 5' end and a SacI site at the 3' end were used in the PCR to generate a 430 bp fragment which contained the TF8-5G9 HC Kozak sequence, start codon, signal sequence, and variable region. This fragment was digested with the restriction enzymes EcoRI and SacI, and gel-purified using the same procedure that was used with the chimeric LC construction.

The full-length TF8-5G9 chimeric HC gene was constructed by cloning the variable region fragment into the EcoRI and SacI sites of the pSG5 expression vector containing the human IgG4 constant region.

EXAMPLE 3

Design and Construction of the CDR-Grafted Heavy and Light Chain Genes

The variable region domains of the CDR-grafted HC and LC genes were designed with an EcoRI overhang at the 5' end followed by a Kozak sequence to improve antibody expression. The leader sequences were derived from the heavy and light chains of the murine monoclonal antibody B72.3 (Whittle et al. (1987) Protein Engineering 1:499). The 3' end of the variable regions were designed to have overhangs which allowed for splicing to the appropriate human constant region DNA.

In the initially designed CDR-grafted TF8-5G9 heavy and light chains the CDRs were derived from murine TF8-5G9 sequence while the frameworks were derived primarily from human antibody sequence. The human antibody KOL (Schmidt et al.) was used for the heavy chain frameworks, while the human antibody dimer (Epp et al.) was used for the light chain frameworks.

Several criteria were used to select murine framework residues in the design of the TF8-5G9 CDR-grafted heavy and light chain variable regions. Framework residues which, at a particular position, are idiosyncratic to TF8-5G9 were retained as murine sequence with the assumption that they contributed to its unique binding characteristics. TF8-5G9 murine residues were also retained at framework positions where they were in agreement with the human consensus sequence but where the corresponding residues in KOL or REI were idiosyncratic. Residues that are part of antibody loop canonical structures such as residue 71 (numbering

according to Kabat et al.) of the heavy and light chains were also retained as murine sequence. Framework residues that form loops such as residues 26-30 of the HC were kept as TF8-5G9 murine sequence at positions were the murine sequence differed from the human. Residues known to directly influence the conformation of CDRs, such as 48 and 49 immediately preceding CDR2 of the HC, were also retained as murine sequence.

The amino acid sequence of the variable region for the initially designed CDR-grafted TF8-5G9 HC, TF8HCDR1, is shown in SEQ ID NO:11. Murine residues were retained at framework positions 6, 17, 23, 24, 28, 29, 30, 48, 49, 68, 71, 73, 78 88 and 91. The CDR-grafted HC variable region was attached to a human IgG4 constant region.

The amino acid sequence of the variable region for the initially designed CDR-grafted TF8-5G9 LC, TF8LCDR1, is shown in SEQ ID NO:12. Murine residues were retained at framework positions 39, 41, 46 and 105. The CDR-grafted LC variable region was attached to a human kappa constant region.

The variable region for the CDR-grafted HC and LC described above were each assembled from 13 synthetic oligonucleotides which were synthesized by Research Genetics, Inc., Huntsville, AL. These oligonucleotides ranged in length from 42 to 80 bases, and encoded both variable region strands. When the 6 complementary oligonucleotide pairs were annealed, the overhangs generated were 17 to 24 bases in length. These oligonucleotide pairs were combined, annealed at their complementary overhangs, and ligated to give the final full length double-stranded variable regions.

The HC variable region oligonucleotides were assembled into a 452 bp fragment which contains a 5' EcoRI site and a 3' SacI site. The polymerase chain reaction was used to amplify this fragment. The resulting amplified DNA was purified on a 2% Nusieve, 1% Seakem agarose gel (FMC). The appropriate size band of DNA was excised and the DNA was recovered by the Geneclean (Bio 101) procedure. The fragment was then digested with EcoRI and SacI, and purified again by the Geneclean method. This HC variable region fragment with EcoRI and SacI ends was cloned into the EcoRI and SacI sites of the pSport-1 vector (GIBCO-BRL Life Technologies, Gaithersburg, MD). DNA from several clones was isolated and sequenced to verify proper variable region assembly. All clones had unexpected base changes. One clone with the fewest base changes (two mismatches at bases 133 and 140) was selected to be corrected by site-directed mutagenesis according to Kunkel (1985) Proc. Natl. Acad. Sci. USA 82:488. Briefly, CJ236 (ung-, dut-) competent cells (Invitrogen Corporation, San Diego, CA) were transformed with the pSport vector containing the CDR-grafted HC variable region with the two base mismatch. Single-stranded, uridine-incorporated DNA templates were purified from phage following M13 helper phage (Stratagene Cloning Systems) infection of the transformed cells. Mutagenesis oligos containing the desired base changes were synthesized on an Applied Biosystems Model 380B DNA synthesizer. The mutagenesis oligos were annealed to the template DNA, and T7 DNA Polymerase and T4 DNA Ligase (MutaGene InVitro Mutagenesis Kit, Bo-Rad Laboratories, Richmond, CA) were used to incorporate the

oligo into a newly synthesized DNA strand. DH5α competent cells (GIBCO-BRL Life Technologies) were transformed with the double-stranded DNA. The original uridine-incorporated strand is destroyed while the newly synthesized strand containing the mutagenesis oligo is replicated. Phagemid DNA was prepared from the resulting mutagenesis clones and the variable regions were sequence to identify the clones which had incorporated the desired changes. The corrected HC EcoRI/SacI variable region fragment was excised from the pSport vector, purified and ligated into the EcoRI/SacI sites of a pSG5 vector containing the human IgG4 constant region. This resulted in the generation of a full-length humanized TF8-5G9 HC gene, TF8HCDR1, in the pSG5 COS cell expression vector. The vector was designated pSG5TF8HCDR1.

The CDR-grafted TF8-5G9 LC variable region was also amplified by the PCR from the assembled synthetic oligonucleotides into a 433 bp fragment which contained a 5' EcoRI site and a 3' NarI site. This fragment was purified as described above for the HC, digested with EcoRI and NarI and purified by the Geneclean procedure. This fragment was cloned into the EcoRI and NarI sites of a pSG5 vector which contains the human kappa constant region. This resulted in the generation of a full-length humanized TF8-5G9 LC gene, TF8LCDR1, in the pSG5 COS cell expression vector. Seven clones were sequenced, and one was found to have the desired CDR-grafted LC sequence. The vector was designated pSQ5TF8LCDR1.

EXAMPLE 4

Expression of the CDR-Grafted Heavy and Light Chain Genes in COS Cells

The transient expression of antibody genes in COS-1 cells provides a rapid and convenient system to test antibody gene expression and function. COS-1 cells were obtained from the American Type Culture Collection (CRL 1650) and cultured in Dulbecco's Modified Eagle Medium (DMEM, from GIBCO BRL Life Technologies) with 10% fetal calf serum. The pSG5TF8HCDR1 expression factor was cotransfected into COS cells with the pSG5 chimeric LC expression vector using the DEAE-Dextran method followed by DMSO shock as described by Lopata et al. (1984) Nucleic Acids Res. 14:5707. After 4 days of culture, media was harvested from the wells and examined for antibody expression levels.

Antibody levels were determined by an ELISA-based assembly assay. Plates were coated with a goat anti-human Fc specific antibody. Various dilutions of the COS cell supernatant containing secreted antibody were added, incubated for one hour, and washed. A horseradish peroxidase-linked goat anti-human kappa chain antibody was added, incubated for one hour at room temperature, and washed. Substrate for the horseradish peroxidase was added for detection. Antibody levels in the COS cell media were found to be nearly undetectable for the TF8HCDR1 x chimeric LC. Upon closer examination of the TF8HCDR1 variable region sequence, it was found that an unexpected base change, which had occurred during the site-directed mutagenesis process described in Example 3, introduced a stop codon into framework 4

of the TF8HCDR1 gene. This substitution was corrected by site-directed mutagenesis as described above. Thorough sequencing of the variable region confirmed that the correction was made with no additional changes introduced. Upon transfection of this corrected TF8HCDR1 gene with the chimeric LC, reasonable expression levels were obtained.

COS cells which had been co-transfected with the CDR-grafted LC expression vector, pSGTF8LCDR1, and either the chimeric HC or TF8HCDR1, produced antibody at reasonable levels. Antibody levels in COS cell supernatants ranged from 0.5 μg to 10.0 μg per ml.

EXAMPLE 5

Binding of the CDR-Grafted TF8-5G9 to Tissue Factor

An ELISA was used to determine the ability of the CDR-grafted TF8-5G9 antibody, TF8HCDR1 x TF8LCDR1, to bind to tissue factor. Tissue factor was immobilized on a microtiter plate. The test COS cell supernatant, containing the CDR-grafted antibody, was added to the well, incubated for one hour at room temperature. Following three washes with PBS/Tween, a goat anti-human kappa chain polyclonal antibody conjugated to horseradish peroxidase was added, incubated for one hour at room temperature and washed. Substrate for the horseradish peroxidase was added for detection. The positive control was the TF8-5G9 chimeric antibody. The CDR-grafted TF8-5G9 antibody was able to bind to tissue factor to a degree comparable to the chimeric TF8-5G9 antibody (Figure 3, solid symbols).

The ability of the humanized antibody to compete with murine TF8-5G9 for binding to tissue factor was also examined. Varying amounts of COS cell supernatant containing the test CDR-grafted antibody and a fixed amount of murine TF8-5G9 were added simultaneously to wells coated with tissue factor. Binding was allowed to occur for one hour at room temperature. The wells were washed three times with PBS/Tween. A goat anti-human kappa chain antibody conjugated to horseradish peroxidase was added, incubated for one hour at room temperature and washed. Substrate for the horseradish peroxidase was added for detection. The positive antibody competed as well as

the chimeric antibody with murine TF8-5G9 for binding to TF.

These data indicate that the initially designed CDR-grafted antibody, TF8HCDR1 x TF8LCDR1, was approximately as active as the chimeric TF8-5G9 in binding to TF and competing with the murine antibody for binding to TF.

EXAMPLE 6

Construction and Characterization of Additional CDR-Grafted Heavy Chains

Upon examination of the molecular structure of murine TF8-5G9, framework residues at positions 27, 68, 73 and 78 were found to lie on the antibody surface and had no discernible contact with the CDRs. These framework residues were of murine sequence in TF8HCDR1 but were changed to the human KOL sequence in various combinations to generate a series of CDR-grafted heavy chains with framework residue variations. The changes were made by the process of site-directed mutagenesis as described in Example 3. Each CDR-grafted heavy chain version was expressed in COS cells in combination with the CDR-grafted LC, TF8LCDR1, and tested for its ability to bind TF and compete with murine TF8-5G9 for binding. Every version of the CDR-grafted heavy chain in combination with TF8LCDR1 was shown to bind TF with an affinity comparable to chimeric TF8-5G9. Every CDR-grafted HC in combination with TF8LCDR1 was able to compete with murine TF8-5G9 for binding to TF to a degree comparable to the chimeric antibody.

Changes in sequence from murine to human for HC framework positions 6, 7, 68, 73 and 78 did not adversely affect the antigen binding ability of the antibody. The CDR-grafted HC version which had human sequence at all of these positions, and thus was the most humanized HC, was TF8HCDR20.

The complete sequence of the TF8HCDR20 gene was determined. The DNA sequence is shown as a 2360 bp EcoRI/BamHI insert with protein translation in the

pEe6TF8HCDR20 expression vector in Figure 4 and SEQ ID NO:15.

The essential regions of the gene are as follows:

Nucleotide #	Region
1-6	5' EcoRI restriction site
7-15	Kozak sequence
16-72	Start codon and leader sequence
73-423	CDR-grafted variable region
424-717	Human IgG4 CH1 domain
718-1110	Human IgG4 intron 2
1111-1146	Human IgG4 hinge
1147-1267	Human IgG4 intron 3
1268-1594	Human IgG4 CH2 domain
1595-1691	Human IgG4 intron 4
1692-2012	Human IgG4 CH3 domain
2013-2354	3' untranslated region
2355-2360	3' BamHI end spliced to BclI site of the expression vector

EXAMPLE 7

C nstruction and Characterization of Additional CDR-Grafted Light Chains

The initially designed CDR-grafted LC, TF8LCDR1, contained four framework residues from the murine TF8-5G9 sequence. At two of these positions, 39 and 105, the human REI framework sequence is unique to REI; however, the murine TF8-5G9 LC sequence is in agreement with the human consensus sequence. The other two murine framework residues, trp41 and thr46, are unique to TF8-5G9. Several versions of the CDR-grafted LC were generated in which the sequence at these four positions were changed from the murine to the human REI in various combinations. These changes were made by site-directed mutagenesis. Each version of the CDR-grafted LC was expressed in COS cells in combination with the CDR-grafted HC, TF8HCDR20, and tested for ability to bind tissue factor and compete with murine TF8-5G9 for binding. Every version of the CDR-grafted LC, in combination with TF8HCDR20, was shown to bind TF with an affinity comparable to TF8-5G9. Also every CDR-grafted LC version, in combination with TF8HCDR20, was able to compete with murine TF8-5G9 for binding to TF in a manner comparable to the chimeric TF8-5G9 control.

Changes in sequence from murine to human for LC framework positions 39, 41, 46 and 105 did not adversely effect the ability of the antibody to recognize antigen. The CDR-grafted LC of choice was TF8LCDR3, where murine TF8-5G9 sequence was used at positions 39 and 105 because these are in agreement with

the human consensus sequence. The preferred CDR-grafted TF8-5G9 antibody is TF8HCDR20 x TF8LCDR3.

The complete sequence of the TF8LCDR3 gene was determined and is shown as a 759 bp EcoRI-BamHI insert with protein translation in the pEe12TF8LCDR3 expression vector in Figure 5 and SEQ ID NO:17. The essential regions of the gene are as follows:

Nucleotide #	Region
1-5	5' EcoRI restriction site
6-8	Kozak sequence
9-68	Start codon and leader sequence
69-392	CDR-grafted variable region
393-710	Human kappa constant region
711-753	3' untranslated region
754-759	3'BamHI end spliced to BclI site of the expression vector

EXAMPLE 8

CDR-Grafted TF8-5G9 Antibody TF8HCDR20 x TF8LCDR3 Inhibits Human Tissue Factor

The binding of the CDR-grafted TF8-5G9 antibody, TF8HCDR20 x TF8LCDR3, to TF was assessed as described in Example 5 and was found to be comparable to that of the chimeric TF8-5G9 as illustrated in Figure 6. The ability of the CDR-grafted TF8-5G9 to compete with the murine antibody for binding to TF is comparable to that of the chimeric TF8-5G9 as shown in Figure 7.

An in vitro assay was used to measure the level of inhibition of factor X activation by the CDR-grafted TF8-5G9 antibody. In this assay, TF forms an active proteolytic complex with factor VII. This complex then converts factor X to factor Xa by proteolysis. The activated Xa enzymatically cleaves a substrate, Spectrozyme FXa, which releases a chromogen. The level of chromogen, as detected by optical density, is an indication of factor X activation due to TF-factor VIIa activity.

The following reaction mixtures were prepared in 12 x 75 mm borosilicate glass tubes.

25 μl TBS (50 mM Tris, pH 7.4, 150 mM NaCl)

15 μl 20 mM $CaCl_2$/1% bovine serum albumin (BSA)

20 μl human placental tissue factor solution (prepared by reconstituting one vial of Thromborel S, Curtin Matheson Scientific #269-338 with 4.0 ml dH_2O and diluting 1:10 in TBS)

30 μl Factor VII (Enzyme Research Labs #HFVII 1007 at 237.66 ng/ml in TBS)

30 μl TBS or TF8-5G9 or TF8MCDR20 x TF8LCDR3 at 1.18 μg/ml or as indicated in Fig. 8

The reaction mixtures were incubated at 37°C for ten minutes before the addition of Factor X. (In some cases the reaction mixture was preincubated for five minutes before addition of Factor VII or antibody, followed by a ten minute incubation before addition of Factor X.) Thirty μl of Factor X solution (Enzyme Research Labs, DHFX 330, 247.38 μg/ml TBS) was added and the mixture was incubated at 37°C for three minutes. Factor X activation was terminated by pipetting 40 μg of reaction mixture into 160 μl of stop buffer (50 mM Tris, pH 7.4, 100 mM EDTA, 150 mM NaCl) in 96 well microtiter plates. Each tube of reaction mixture was pipetted into three microtiter wells. Fifty μl of Spectrozyme FXa substrate (American Diagnostica #222, 1μM/ml TBS) was added to each well. OD_{405} was read on a Molecular Devices kinetic plate reader with readings taken every twenty seconds for ten minutes. Factor X activity was recorded as mOD/minute, and enzyme velocities over the linear portion of the reaction curve were compared to determine inhibition of factor X activation by the anti-TF antibodies.

As shown in Figure 8, the CDR-grafted TF8-5G9 antibody is approximately as effective as the murine TF8-5G9 in inhibiting factor X activation. This indicates that the CDR-grafted TF8-5G9 is functionally active.

EXAMPLE 9

C nstruction of the CDR-Grafted Heavy and Light Chain Myeloma Expression Vectors

For the purpose of establishing a permanent CDR-grafted antibody-producing cell line, the TF8HCDR20 and TF8LCDR3 genes were subcloned into myeloma cell expression vectors. The heavy chain TF8HCDR20 was subcloned into the EcoRI and BclI sites of the pEe6hCMV-BglII myeloma expression vector described by Stephens et al. (1989) Nucleic Acids Res. 17:7110 to produce pEe6TF8HCDR20. The light chain TF8LCDR3 was subcloned into the EcoTI and BclI sites of the pEe12 myeloma expression vector to produce pEe12TF8LCDR3. The heavy and light chain expression vectors are illustrated in Figures 9 and 10, respectively. In both vectors antibody gene transcription was driven by the human cytomegalovirus (hCMV) promoter-enhancer, which lies directly 5' to the multiple cloning site. The polyadenylation signal sequence lies 3' to the multiple cloning site and signals the termination of transcription. Each vector contains the ß-lactamase gene to allow for ampicillin selection in E. coli. The pEe12 vector contains a glutamine synthetase cDNA gene under the transcriptional control of the SV40 early promoter. Glutamine synthetase allows for myeloma cell transfectants to be selected in glutamine-free media. Myeloma cells are devoid of glutamine synthetase activity and are dependent on a supply of glutamine in the culture media. Cells which have been transfected with the pEe12 vector, containing the glutamine

synthetase gene, are able to synthesize glutamine from glutamate and can survive in the absence of glutamine.

The pEe6TF8HCDR20 expression vector is a 7073 bp plasmid whose DNA sequence is shown in Figure 4 and SEQ ID NO:15. The coding regions of the TF8HCDR20 gene are translated. The essential regions of this vector are described below:

1. Nucleotides #1-2360: The TF8HCDR20 CDR-grafted HC gene is described in Example 6. The HC gene was inserted as an EcoRI/BamHI fragment into the EcoRI/BclI sites of the pEe6hCMV-BglII vector.

2. Nucleotides #2361-2593: This region encodes the SV40 early gene polyadenylation signal (SV40 nucleotides 2770-2537), which acts as a transcriptional terminator. This fragment is flanked by a 5' BclI site and a 3' BamHI site. The 3' BamHI end of the heavy chain gene was spliced to the 5' BclI site of the polyadenylation signal, thus eliminating both sites.

3. Nucleotides #2594-3848: This region is a BamHI-BglI fragment from pBR328 (nucleotides 375-2422) but with a deletion between the SaI and AvaI sites (pBR328 nucleotides 651-1425) following the addition of a SalI linker to the AvaI site. This region contains the Col E1 bacterial origin of replication.

4. Nucleotides #3849-4327: This is a BglI-XmnI fragment site from the ß-lactamase gene of pSP64 (Promega Corporation, Madison, WI). This gene provides ampicillin resistance to bacteria transformed with this vector.

5. Nucleotides #4328-4885: This is an XmnI-HindIII fragment of the ColE1 based plasmid pCT54 described by Emtage et al. (1983) Proc. Natl. Acad. Sci. USA

80:3671. The HindIII site was converted to a BglII site by the addition of a linker following the addition of the hCMV promoter described below.

6. Nucleotides #4886-7022: These nucleotides encode the Pst-1m fragment of human cytomeglovirus (hCMV) strain AD 169 described by Greenway et al. (1982) Gene 18:355 containing the region coding for the hCMV middle intermediate early promoter. This Pst-1m fragment was cloned into the HindIII site of pEe6hCMV by addition of oligonucleotides of the following sequence to either end of the fragment:

5' GTCACCGTCCTTGACACGA 3'

3' ACGTCAGTGGCAGGAACTGTGCTTCGA 5'

The resulting 2100 bp fragment was inserted such that the promoter directed transcription towards the EcoRI site of pEe6hCMV. The oligonucleotide above served to recreate the complete 5' untranslated sequence of the hCMV-MIE gene the added irrelevant sequence at the very 5' end of the fragment. The HindIII site at the 5' end was subsequently converted to a BglII site by the addition of a further linker.

7. Nucleotides #7023-7073: The pSP64 polylinker with the BamHI and SaII sites removed.

The pEe12TF8LCDR3 expression vector is a 7864 bp plasmid whose DNA sequence is shown in Figure 5 and SEQ ID NO:17. The coding regions of the TF8LCDR3 gene are translated. The essential regions of this expression vector are described below:

1. Nucleotides #1-759: The TF8LCDR3 CDR-grafted LC gene is described in Example 7. The gene was inserted as an

EcoRI/BamHI fragment into the EcoRI/BclII sites of the pEe12 expression vector.

2. Nucleotides #760-3284: These regions of pEe12 are identical to the regions encoded by nucleotides 2361-4885 of the pEe6TF8HCDR20 vector described above (regions #2-5).

3. Nucleotides #3285-5736: This region encodes the Chinese hamster ovary glutamine synthetase cDNA under the transcriptional control of the SV40 early promoter and followed by the SV40 polyadenylation and splice signals from the pSV2.dhfr vector described by Subramani et al. (1981) Mol. Cell. Biol. 1:854. The following describes the derivation of this region: A 1200 bp NaeI-PvuII fragment, containing a complete GS coding sequence, was excised from the Chinese hamster ovary cDNA clone λGS1.1 described by Hayward et al. (1986) Nucleic Acid Res. 14:999. After addition of a HindIII linker to the NaeI site and a BglII linker to the PvuII site (hence destroying the NaeI and PvuII sites), the 1200 bp fragment was cloned in place of DHFR sequences in pSV2.dhfr between the HindIII and BglII sites to form pSV2.GS. The single remaining PvuII site in pSV2BamGS was converted to a BamHI site by addition of an oligonucleotide linker to form pSV2BamGS. An EcoRI site in the GS cDNA was destroyed by site directed mutagenesis without altering the amino acid sequence in pSV2BamGS and the HindIII site was destroyed by filling in with DNa polymerase I. The 2451 bp BamHI fragment from this plasmid, containing the complete SV40-GS hybrid transcription unit, was excised and inserted at the BglII site of pEe6hCMV-BglII site of pEe6hCMV-BglII such that transcription from the sV40 early promoter proceeds towards the hCMV promoter.

4. Nucleotides #5737-7864: This region is identical to the hCMV promoter and pSP64 polylinker encoded by nucleotides 4886-7073 of the pEe6TF8HCDR20 vector described above (regions 6 and 7).

For the purpose of ensuring that both the pEe6TF8HCDR20 and peE12TF8LCDR3 vectors co-transfected myeloma cells, the vectors were joined in linear concatamers. Both the pEe6TF8HCDR20 and pEe12TF8LCDR3 vectors were digested at the unique SalI site. The SalI linearized pEe6TF8HCDR20 vector was phosphatased at its 5' ends to prohibit ligation of two pEe6TF8HCDR20 vectors onto each other. This phosphatased HC vector was ligated in a 2:1 molar ratio to the Sal linearized pEe12TF8LCDR3. The resulting concatamers were most likely of the following composition:

SalI SalI SalI SalI

| pEe6TF8HCDR20 | pEe12TF8LCDR3 | pEe6TF8HCDR20 |

This concatamerized DNA was extracted with phenol and chloroform, and precipitated with ammonium acetate and ethanol. The DNA precipitate was resuspended in distilled water to a concentration of 1 μg/μL and used to transfect myeloma cells.

EXAMPLE 10

Development of NSO Expression Cell Lines

Stably transformed cell lines expressing the humanized TF8-5G9 antibody were prepared by transfecting CDR-grafted heavy and light chain expression vectors into NSO mouse myeloma cells. Selection of transfected cells was carried out using the dominant selectable marker gene, glutamine synthetase (GS).

The NSO mouse myeloma cell line, obtained from Celltech, Ltd., is a subclone derived from NS-1 and does not express intracellular light chains. These cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with added glutamine and 10% fetal bovine serum (FBS). To prepare for transfection, the cells were harvested in mid-log phase of the growth cycle, centrifuged for 5 minutes, washed with phosphate buffered saline (PBS), centrifuged again, and the cell pellet was resuspended in 2.2 mL of PBS. The final cell concentration was 2.18 x 10^7 mL. Cells were maintained on ice during the entire procedure.

The DNA to be transfected (pEe12TF8LCDR3 x pEe6TF8HCDR20) was prepared as a concatamer as described in Example 9. The DNA and NSO cells were added to a 0.4 cm BioRad Gene Pulser cuvette in the following order:

40 μL (40 μg) DNA concatamer
320 μL double distilled water
40 μL 10 x PBS
400 μL NSO cells (8.72 x 10^6 cells)

Transfection was performed by electroporation following a protocol provided by Celltech, Ltd. In this procedure, the cells and DNA in PBS buffer were exposed

to a brief, high voltage pulse of electricity causing transient micropores to form on the cell membrane. DNA transfer takes place through these openings. To prepare for electroporation, the suspension of NSO cells and DNA was gently mixed and incubated on ice for 5 minutes. The cuvette was placed in a BioRad Gene Pulser and given 2 consecutive electrical pulses at settings of 3 μF (capacitance) and 1.5V (voltage). Following electroporation, the cuvette was returned to the ice for 5 minutes. The suspension was then diluted in prewarmed growth medium and distributed into seven 96-well plates. Control plates containing cells electroporated without DNA were also prepared at the same time to measure the presence of spontaneous mutants. Plates were placed in a 37°C incubator with 5% CO_2.

Glutamine synthetase, encoded by the GS gene, is an enzyme that converts glutamate to glutamine. NSO cells require glutamine for growth due to inadequate levels of endogenous GS gene expression. In the DNA concatamer, this gene is located on the pEe12TF8LCDR3 vector. Transfected cells which incorporate the GS gene become glutamine-independent. Cells not integrating the GS gene into their genome would remain glutamine-dependent and would not survive in glutamine-free medium. Approximately 18 hours post electroporation, all plates were fed with glutamine-free selection medium and returned to the incubator until viable colonies appeared.

Approximately 3 weeks after transfection, distinct macroscopic colonies were observed. These were screened for expression of the intact humanized antibody using the assembly ELISA as described in Example 5.

Tissue culture supernatants from wells containing colonies were screened at a 1:10 dilution. Positive wells showing activity greater than the 25 ng/mL standard were subcultured and expanded for further analysis.

For selection of high producers, antibody production was quantitated after a 96 hour growth period. Tissue culture flasks were seeded with 2 x 10^5 cells/mL in 10 mL of selection medium and incubated at 37°C, 5% CO_2 for 96 hours. At the end of that time period, an aliquot was taken to determine cell concentration and antibody titer. Evaluation of antibody production was calculated as μg/mL and pg/cell/96 hours. The highest producers from this transfection were:

Cell Line	μg/mL	pg/cell/96 hour
2B1	26.3	24.3
3E11	27.6	59.9
4G6	30.2	41.9

EXAMPLE 11

CDR Grafted Antibody TF8HCDR20 x TF8LCDR3 Inhibits Tissue Factor In Vivo

CDR grafted antibody TF8HCDR20 x TF8LCDR3 was compared to murine antibody TF8-5G9 for its ability to protect rats from experimentally induced disseminated intravascular coagulation (DIC). In the DIC model, rats are challenged with human thromboplastin (a crude tissue extract containing TF activity), resulting in fibrinogen consumption and death. Pretreatment of rats with anti-TF antibody was demonstrated to protect rats from fibrinogen consumption and death as follows.

Human thromboplastin was prepared as described in U.S. Patent 5,223,427. Saline control or 30 μ/ml of TF8-5G9 or CDR-grafted antibody was injected through the tail vein of rats, followed by injection of thromboplastin equivalent to 200 ng of recombinant TF. Clotting times were determined at T=0 and T=1 minute as a measure of fibrinogen concentration. Clotting times are proportional to fibrinogen concentration, with a 60 second clotting time corresponding to an 80% reduction in fibrinogen concentration. Clotting times of greater than 60 seconds cannot be accurately measured and were recorded as 60 seconds.

Survivability and clotting times for three representative studies are shown below.

Survivors

Study	Controls	TF8-5G9	CDR-grafted Ab
1	0/8	5/8	6/8
2	0/8	4/7	7/8
3	0/8	8/8	3/7

Clotting Times
Controls

Study #1 T=0	Study #1 T=1	Study #2 T=0	Study #2 T=1	Study #3 T=0	Study #3 T=1
16	>60	18	>60	19	>60
16	>60	18	>60	21	>60
16	>60	18	>60	18	>60
17	>60	18	>60	19	>60
15	>60	16	>60	18	54
16	>60	18	>60	18	>60
16	>60	17	>60	18	>60
16	>60	17	>60	18	>60

Clotting Times
Murine TF8-5G9

Study #1 T=0	Study #1 T=1	Study #2 T=0	Study #2 T=1	Study #3 T=0	Study #3 T=1
16	36	18	34	19	28
15	41	18	36	18	29
15	33	18	>60	19	29
15	31	17	>60	18	29
15	>60	18	50	18	28
16	>60	17	34	19	40
16	33	17	34	19	40
16	33	18	31	19	34
16	>60			19	>60

Clotting Times
CDR-grafted TF8-5G9

Study #1 T=0	Study #1 T=1	Study #2 T=0	Study #2 T=1	Study #3 T=0	Study #3 T=1
16	>60	17	>60	21	>60
16	>60	17	33	18	34
16	>60	18	32	17	>60
22	37	18	>60	20	35
16	32	17	32	17	58
15	>60	18	31	18	33
16	>60	17	31	18	31
16	>60	16	32		

Twenty-three of the twenty-four control rats had clotting times of greater than 60 seconds indicating that virtually all untreated rats were consuming more than 80% of their fibrinogen. Both the CDR-grafted and murine antibody treated rats had similar clotting times at one minute of 44.5 and 40 seconds. Further, only six of the murine antibody treated rats and nine of the CDR-grafted antibody treated rats had clotting times in excess of 60 seconds. Accordingly, both the murine and CDR-grafted antibodies were able to neutralize TF and thus protect rats from fibrinogen consumption and death.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Joliffe, Linda K.
Zivin, Robert A.
Pulito, Virginia L.

(ii) TITLE OF INVENTION: CDR-GRAFTED ANTI-TISSUE FACTOR ANTIBODIES AND METHODS OF USE THEREOF

(iii) NUMBER OF SEQUENCES: 20

(iv) CORRESPONDENCE ADDRESS:
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(F) ZIP: 11530

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 07-JUN-1995
(C) CLASSIFICATION:

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(C) TELEX: 230 901 SANS UR

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1489 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 11..1391

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GTT ACA GGG GTC AAT TCA GAG ATT CAG CTG CAG CAG TCT GGG GCT GAG      97
Val Thr Gly Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu
     15                  20                  25

CTT GTG AGG CCA GGG GCC TTA GTC AAG TTG TCC TGC AAA GCT TCT GGC     145
Leu Val Arg Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly
 30                  35                  40                  45

TTC AAC ATT AAA GAC TAC TAT ATG CAC TGG GTG AAG CAG AGG CCT GAA     193
Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu
                 50                  55                  60

CAG GGC CTG GAG TGG ATT GGA TTG ATT GAT CCT GAG AAT GGT AAT ACT     241
Gln Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr
             65                  70                  75

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         80                  85                  90

TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT GAG GAC     337
Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp
     95                 100                 105

ACT GCC GTC TAT TAC TGT GCT AGA GAT AAC TCG TAC TAC TTT GAC TAC     385
Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr
110                 115                 120                 125
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Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser
            145                 150                 155

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Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val
        160                 165                 170

ACA GTG ACC TGG AAC TCT GGA TCC CTG TCC AGC GGT GTG CAC ACC TTC      577
Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe
    175                 180                 185

CCA GCT GTC CTG CAG TCT GAC CTC TAC ACT CTG AGC AGC TCA GTG ACT      625
Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr
190                 195                 200                 205

GTG CCC TCC AGC ACC TGG CCC AGC GAG ACC GTC ACC TGC AAC GTT GCC      673
Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala
                210                 215                 220

CAC CCG GCC AGC AGC ACC AAG GTG GAC AAG AAA ATT GTG CCC AGG GAT      721
His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp
            225                 230                 235

TGT GGT TGT AAG CCT TGC ATA TGT ACA GTC CCA GAA GTA TCA TCT GTC      769
Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val
        240                 245                 250

TTC ATC TTC CCC CCA AAG CCC AAG GAT GTG CTC ACC ATT ACT CTG ACT      817
Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr
    255                 260                 265

CCT AAG GTC ACG TGT GTT GTG GTA GAC ATC AGC AAG GAT GAT CCC GAG      865
Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu
270                 275                 280                 285

GTC CAG TTC AGC TGG TTT GTA GAT GAT GTG GAG GTG CAC ACA GCT CAG      913
Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln
                290                 295                 300

ACG CAA CCC CGG GAG GAG CAG TTC AAC AGC ACT TTC CGC TCA GTC AGT      961
Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser
            305                 310                 315
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TGC AGG GTC AAC AGT GCA GCT TTC CCT GCC CCC ATC GAG AAA ACC ATC      1057
Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile
    335                 340                 345

TCC AAA ACC AAA GGC AGA CCG AAG GCT CCA CAG GTG TAC ACC ATT CCA      1105
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350                 355                 360                 365

CCT CCC AAG GAG CAG ATG GCC AAG GAT AAA GTC AGT CTG AAC TGC ATG      1153
Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Asn Cys Met
            370                 375                 380

ATA ACA GAC TTC TTC CCT GAA GAC ATT ACT GTG GAG TGG CAG TGG AAT      1201
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            385                 390                 395

GGG CAG CCA GCG GAG AAC TAC AAG AAC ACT CAG CCC ATC ATG GAC ACA      1249
Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr
        400                 405                 410

GAT GGC TCT TAC TTC GTC TAC AGC AAG CTC AAT GTG CAG AAG AGC AAC      1297
Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn
    415                 420                 425

TGG GAG GCA GGA AAT ACT TTC ACC TGC TCT GTG TTA CAT GAG GGC CTG      1345
Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu
430                 435                 440                 445

CAC AAC CAC CAT ACT GAG AAG AGC CTC TCC CAC TCT CCT GGT AAA T        1391
His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys
            450                 455                 460

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CCCTGTATAA ATAAAGCACC CAGCACTGCC TTGGACCC                            1489
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 460 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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         35                  40                  45

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     50                  55                  60

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 65                  70                  75                  80

Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr Ser Ser Asn
                 85                  90                  95

Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln
        115                 120                 125

Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val
    130                 135                 140

Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr
145                 150                 155                 160

Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr
                165                 170                 175

Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val
            180                 185                 190

Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser
        195                 200                 205

Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala
    210                 215                 220
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Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys
225 230 235 240

Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe
245 250 255

Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val
260 265 270

Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe
275 280 285

Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro
290 295 300

Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro
305 310 315 320

Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val
325 330 335

Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
340 345 350

Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys
355 360 365

Glu Gln Met Ala Lys Asp Lys Val Ser Leu Asn Cys Met Ile Thr Asp
370 375 380

Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro
385 390 395 400

Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser
405 410 415

Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala
420 425 430

Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His
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His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 937 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 5..706

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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       1               5                  10                  15

CCA GGT ATC AGA TGT GAC ATC AAG ATG ACC CAG TCT CCA TCC TCC ATG       97
Pro Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met
                20                  25                  30

TAT GCA TCG CTG GGA GAG AGA GTC ACT ATC ACT TGT AAG GCG AGT CAG      145
Tyr Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln
            35                  40                  45

GAC ATT AGA AAG TAT TTA AAC TGG TAC CAG CAG AAA CCA TGG AAA TCT      193
Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser
        50                  55                  60

CCT AAG ACC CTG ATC TAT TAT GCA ACA AGC TTG GCA GAT GGG GTC CCA      241
Pro Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro
    65                  70                  75

TCA AGA TTC AGT GGC AGT GGA TCT GGG CAA GAT TAT TCT CTA ACC ATC      289
Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile
 80                  85                  90                  95

AGC AGC CTG GAG TCT GAC GAT ACA GCA ACT TAT TAC TGT CTA CAA CAT      337
Ser Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His
                100                 105                 110

GGT GAG AGC CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAC      385
Gly Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn
            115                 120                 125
```

```
AGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA TCC AGT GAG        433
Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
        130                 135                 140

CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC TTC TTG AAC AAC TTC        481
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
    145                 150                 155

TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT GAT GGC AGT GAA CGA        529
Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
160                 165                 170                 175

CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG GAC AGC AAA GAC AGC        577
Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
                180                 185                 190

ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC AAG GAC GAG TAT GAA        625
Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
            195                 200                 205

CGA CAT AAC AGC TAT ACC TGT GAG GCC ACT CAC AAG ACA TCA ACT TCA        673
Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
        210                 215                 220

CCC AAT GTC AAG AGC TTC AAC AAG AAT GAG TGT TAGAGACAAA GGTCCTGAGA      726
Pro Asn Val Lys Ser Phe Asn Lys Asn Glu Cys
    225                 230

CGCCACCACC AGCTCCCCAG CTCCATCCTA TCTTCCCTTC TAAGGTCTTG GAGGCTTCCC      786

CACAAGCGAC CTACCACTGT TGCGGTGCTC CAAACCTCCT CCCCACCTCC TTCTCCTCCT      846

CCTCCCTTTC CTTGGCTTTT ATCATGCTAA TATTTGCAGA AAATATTCAA TAAAGTGAGT      906

CTTTGCACTT GAAAAAAAAA AAAAAAAAAA A                                     937
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 234 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe Pro
1 5 10 15

Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr
20 25 30

Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp
35 40 45

Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser Pro
50 55 60

Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser
85 90 95

Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His Gly
100 105 110

Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn Arg
115 120 125

Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln
130 135 140

Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr
145 150 155 160

Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln
165 170 175

Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr
180 185 190

Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg
195 200 205

His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
210 215 220

Asn Val Lys Ser Phe Asn Lys Asn Glu Cys
225 230

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asp Asp Tyr Met His
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Lys Pro Lys Phe Gln
1 5 10 15

Gly

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Asp Asn Ser Tyr Tyr Phe Asp Tyr
1               5
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Lys Ala Ser Gln Asp Ile Arg Lys Tyr Leu Asn
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Tyr Ala Thr Ser Leu Ala Asp
1               5
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Leu Gln His Gly Glu Ser Pro Tyr Thr
1               5
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Leu Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr
            20                  25                  30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp Pro Lys Phe
    50                  55                  60

Gln Gly Arg Phe Ser Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Phe
65                  70                  75                  80
```

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Pro
100 105 110

Val Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Arg Lys Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ala Pro Lys Thr Leu Ile
35 40 45

Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Thr Arg
100 105

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr
            20                  25                  30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp Pro Lys Phe
    50                  55                  60

Gln Gly Arg Phe Thr Ile Ser Ala Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Pro
            100                 105                 110

Val Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Arg Lys Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro Tyr
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7073 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 61..717

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1111..1146

```
(ix) FEATURE:
     (A) NAME/KEY: CDS
     (B) LOCATION: 1268..1594

(ix) FEATURE:
     (A) NAME/KEY: CDS
     (B) LOCATION: 1692..2012

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAATTCGCCT CCACCATGGA ATGGAGCTGG GTCTTTCTCT TCTTCTTGTC AGTAACTACA       60

GGT GTA CAC TCA CAA GTT CAG CTG GTG GAG TCT GGA GGA GGA GTA GTA        108
Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val
  1               5                  10                  15

CAA CCT GGA AGG TCA CTG AGA CTG TCT TGT AAG GCT AGT GGA TTC AAT        156
Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn
             20                  25                  30

ATC AAG GAC TAT TAT ATG CAC TGG GTC AGA CAA GCT CCT GGA AAA GGA        204
Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly
         35                  40                  45

CTC GAG TGG ATA GGT TTA ATT GAT CCT GAG AAT GGT AAC ACG ATA TAT        252
Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr
     50                  55                  60

GAT CCC AAG TTC CAA GGA AGA TTC ATA ATT TCT GCA GAC AAC TCT AAG        300
Asp Pro Lys Phe Gln Gly Arg Phe Ile Ile Ser Ala Asp Asn Ser Lys
 65                  70                  75                  80

AAT ACA CTG TTC CTG CAG ATG GAC TCA CTC AGA CCT GAG GAT ACA GCA        348
Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala
                 85                  90                  95

GTC TAC TTT TGT GCT AGA GAT AAC AGT TAT TAC TTC GAC TAC TGG GGC        396
Val Tyr Phe Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly
            100                 105                 110

CAA GGA ACA CCA GTC ACC GTG AGC TCA GCT TCC ACC AAG GGC CCA TCC        444
Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
        115                 120                 125

GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC GAG AGC ACA GCC        492
Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
    130                 135                 140
```

```
GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG      540
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145                 150                 155                 160

TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT      588
Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                165                 170                 175

GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG      636
Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190

CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC AAC GTA GAT CAC      684
Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His
        195                 200                 205

AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GGTGAGAGGC CAGCACAGGG     737
Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val
    210                 215

CAGGGAGGGT GTCTGCTGGA AGCCAGGCTC AGCCCTCCTG CCTGGACGCA CCCCGGCTGT     797

GCAGCCCCAG CCCAGGGCAG CAAGGCATGC CCCATCTGTC TCCTCACCCG GAGGCCTCTG     857

ACCACCCCAC TCATGCTCAG GGAGAGGGTC TTCTGGATTT TTCCACCAGG CTCCGGGCAG     917

CCACAGGCTG GATGCCCCTA CCCCAGGCCC TGCGCATACA GGGGCAGGTG CTGCGCTCAG     977

ACCTGCCAAG AGCCATATCC GGGAGGACCC TGCCCCTGAC CTAAGCCCAC CCCAAAGGCC    1037

AAACTCTCCA CTCCCTCAGC TCAGACACCT TCTCTCCTCC CAGATTCGAG TAACTCCCAA    1097

TCTTCTCTCT GCA GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA       1146
               Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro
                1               5                  10

GGTAAGCCAA CCCAGGCCTC GCCCTCCAGC TCAAGGCGGG ACAGGTGCCC TAGAGTAGCC    1206

TGCATCCAGG GACAGGCCCC AGCCGGGTGC TGACGCATCC ACCTCCATCT CTTCCTCAGC    1266

A CCT GAG TTC CTG GGG GGA CCA TCA GTC TTC CTG TTC CCC CCA AAA        1312
  Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
   1               5                  10                  15

CCC AAG GAC ACT CTC ATG ATC TCC CGG ACC CCT GAG GTC ACG TGC GTG      1360
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
                20                  25                  30
```

```
GTG GTG GAC GTG AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG TAC     1408
Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
                35                  40                  45

GTG GAT GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG     1456
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
        50                  55                  60

CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC ATG CAC     1504
Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Met His
    65                  70                  75

CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA     1552
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
 80                  85                  90                  95

GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC AAA GCC AAA             1594
Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
                100                 105

GGTGGGACCC ACGGGGTGCG AGGGCCACAT GGACAGAGGT CAGCTCGGCC CACCCTCTGC   1654

CCTGGGAGTG ACCGCTGTGC CAACCTCTGT CCCTACA GGG CAG CCC CGA GAG CCA    1709
                                         Gly Gln Pro Arg Glu Pro
                                          1               5

CAG GTG TAC ACC CTG CCC CCA TCC CAG GAG GAG ATG ACC AAG AAC CAG     1757
Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
            10                  15                  20

GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC     1805
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
        25                  30                  35

GTG GAG TGG GAG AGT AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG     1853
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
    40                  45                  50

CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AGG CTA     1901
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
 55                  60                  65                  70

ACC GTG GAC AAG AGC AGG TGG CAG GAG GGG AAT GTC TTC TCA GTC TCC     1949
Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Val Ser
                75                  80                  85
```

GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACA CAG AAG AGC CTC TCC 1997
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
90 95 100

CTG TCT CTG GGT AAA TGAGTGCCAG GGCCGGCAAG CCCCCGCTCC CCGGGCTCTC 2052
Leu Ser Leu Gly Lys
105

GGGGTCGCGC GAGGATGCTT GGCACGTACC CCGTCTACAT ACTTCCCAGG CACCCAGCAT 2112

GGAAATAAAG CACCCACCAC TGCCCTGGGC CCCTGTGAGA CTGTGATGGT TCTTTCCACG 2172

GGTCAGGCCG AGTCTGAGGC CTGAGTGACA TGAGGGAGGC AGAGCGGGTC CCACTGTCCC 2232

CACACTGGCC CAGGCTGTGC AGGTGTGCCT GGGCCACCTA GGGTGGGGCT CAGCCAGGGG 2292

CTGCCCTCGG CAGGGTGGGG GATTTGCCAG CGTGGCCCTC CCTCCAGCAG CAGGACTCTA 2352

GAGGATCATA ATCAGCCATA CCACATTTGT AGAGGTTTTA CTTGCTTTAA AAAACCTCCC 2412

ACACCTCCCC CTGAACCTGA AACATAAAAT GAATGCAATT GTTGTTGTTA ACTTGTTTAT 2472

TGCAGCTTAT AATGGTTACA AATAAAGCAA TAGCATCACA AATTTCACAA ATAAAGCATT 2532

TTTTTCACTG CATTCTAGTT GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG 2592

GATCCTCTAC GCCGGACGCA TCGTGGCCGG CATCACCGGC GCCACAGGTG CGGTTGCTGG 2652

CGCCTATATC GCCGACATCA CCGATGGGGA AGATCGGGCT CGCCACTTCG GGCTCATGAG 2712

CGCTTGTTTC GGCGTGGGTA TGGTGGCAGG CCCCGTGGCCG GGGGACTGTT GGGCGCCATC 2772

TCCTTGCATG CACCATTCCT TGCGGCGGCG GTGCTCAACG GCCTCAACCT ACTACTGGGC 2832

TGCTTCCTAA TGCAGGAGTC GCATAAGGGA GAGCGTCGAC CTCGGGCCGC GTTGCTGGCG 2892

TTTTTCCATA GGCTCCGCCC CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG 2952

TGGCGAAACC CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG 3012

CGCTCTCCTG TTCCGACCCT GCCGCTTACC GGATACCTGT CCGCCTTTCT CCCTTCGGGA 3072

AGCGTGGCGC TTTCTCAATG CTCACGCTGT AGGTATCTCA GTTCGGTGTA GGTCGTTCGC 3132

TCCAAGCTGG GCTGTGTGCA CGAACCCCCC GTTCAGCCCG ACCGCTGCGC CTTATCCGGT 3192

AACTATCGTC TTGAGTCCAA CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT 3252

GGTAACAGGA TTAGCAGAGC GAGGTATGTA GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG 3312
CCTAACTACG GCTACACTAG AAGGACAGTA TTTGGTATCT GCGCTCTGCT GAAGCCAGTT 3372
ACCTTCGGAA AAAGAGTTGG TAGCTCTTGA TCCGGCAAAC AAACCACCGC TGGTAGCGGT 3432
GGTTTTTTTG TTTGCAAGCA GCAGATTACG CGCAGAAAAA AAGGATCTCA AGAAGATCCT 3492
TTGATCTTTT CTACGGGGTC TGACGCTCAG TGGAACGAAA ACTCACGTTA AGGGATTTTG 3552
GTCATGAGAT TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTTT 3612
AAATCAATCT AAAGTATATA TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT 3672
GAGGCACCTA TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC 3732
GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC AATGATACCG 3792
CGAGACCCAC GCTCACCGGC TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC 3852
GAGCGCAGAA GTGGTCCTGC AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG 3912
GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA 3972
GGCATCGTGG TGTCACGCTC GTCGTTTGGT ATGGCATCAT TCAGCTCCGG TTCCCAACGA 4032
TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC CTTCGGTCCT 4092
CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC TCATGGTTAT GGCAGCACTG 4152
CATAATTCTC TTACTGTCAT GCCATCCGTA AGATGCTTTT CTGTGACTGG TGAGTACTCA 4212
ACCAAGTCAT TCTGAGAATA GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCGTCAACA 4272
CGGGATAATA CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT 4332
TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT GTAACCCACT 4392
CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA GCGTTTCTGG GTGAGCAAAA 4452
ACAGGAAGGC AAAATGCCGC AAAAAAGGGA ATAAGGGCGA CACGGAAATG TTGAATACTC 4512
ATACTCTTCC TTTTTCAATA TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA 4572
TACATATTTG AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA 4632
AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA TAAAAATAGG 4692

CGTATCACGA GGCCCTGATG GCTCTTTGCG GCACCCATCG TTCGTAATGT TCCGTGGCAC 4752
CGACGACAAC CCTCAAGAGA AAATGTAATC ACACTGGCTC ACCTTCGGGT GGGCCTTTCT 4812
GCGTTTATAA GGAGACACTT TATGTTTAAG AAGGTTGGTA AATTCCTTGC GGCTTTGGCA 4872
GCCAAGCTAG AGATCTCTAG CTTCGTGTCA AGGACGGTGA CTGCAGTGAA TAATAAAATG 4932
TGTGTTTGTC CGAAATACGC GTTTTGAGAT TTCTGTCGCC GACTAAATTC ATGTCGCGCG 4992
ATAGTGGTGT TTATCGCCGA TAGAGATGGC GATATTGGAA AAATCGATAT TTGAAAATAT 5052
GGCATATTGA AAATGTCGCC GATGTGAGTT TCTGTGTAAC TGATATCGCC ATTTTTCCAA 5112
AAGTGATTTT TGGGCATACG CGATATCTGG CGATAGCGCT TATATCGTTT ACGGGGGATG 5172
GCGATAGACG ACTTTGGTGA CTTGGGCGAT TCTGTGTGTC GCAAATATCG CAGTTTCGAT 5232
ATAGGTGACA GACGATATGA GGCTATATCG CCGATAGAGG CGACATCAAG CTGGCACATG 5292
GCCAATGCAT ATCGATCTAT ACATTGAATC AATATTGGCC ATTAGCCATA TTATTCATTG 5352
GTTATATAGC ATAAATCAAT ATTGGCTATT GGCCATTGCA TACGTTGTAT CCATATCATA 5412
ATATGTACAT TTATATTGGC TCATGTCCAA CATTACCGCC ATGTTGACAT TGATTATTGA 5472
CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA TAGCCCATAT ATGGAGTTCC 5532
GCGTTACATA ACTTACGGTA AATGGCCCGC CTGGCTGACC GCCCAACGAC CCCCGCCCAT 5592
TGACGTCAAT AATGACGTAT GTTCCCATAG TAACGCCAAT AGGGACTTTC CATTGACGTC 5652
AATGGGTGGA GTATTTACGG TAAACTGCCC ACTTGGCAGT ACATCAAGTG TATCATATGC 5712
CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT TATGCCCAGT 5772
ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA CGTATTAGTC ATCGCTATTA 5832
CCATGGTGAT GCGGTTTTGG CAGTACATCA ATGGGCGTGG ATAGCGGTTT GACTCACGGG 5892
GATTTCCAAG TCTCCACCCC ATTGACGTCA ATGGGAGTTT GTTTTGGCAC CAAAATCAAC 5952
GGGACTTTCC AAAATGTCGT AACAACTCCG CCCCATTGAC GCAAATGGGC GGTAGGCGTG 6012
TACGGTGGGA GGTCTATATA AGCAGAGCTC GTTTAGTGAA CCGTCAGATC GCCTGGAGAC 6072
GCCATCCACG CTGTTTTGAC CTCCATAGAA GACACCGGGA CCGATCCAGC CTCCGCGGCC 6132

GGGAACGGTG CATTGGAACG CGGATTCCCC GTGCCAAGAG TGACGTAAGT ACCGCCTATA 6192
GAGTCTATAG GCCCACCCCC TTGGCTTCTT ATGCATGCTA TACTGTTTTT GGCTTGGGGT 6252
CTATACACCC CCGCTTCCTC ATGTTATAGG TGATGGTATA GCTTAGCCTA TAGGTGTGGG 6312
TTATTGACCA TTATTGACCA CTCCCCTATT GGTGACGATA CTTTCCATTA CTAATCCATA 6372
ACATGGCTCT TTGCCACAAC TCTCTTTATT GGCTATATGC CAATACACTG TCCTTCAGAG 6432
ACTGACACGG ACTCTGTATT TTTACAGGAT GGGGTCTCAT TTATTATTTA CAAATTCACA 6492
TATACAACAC CACCGTCCCC AGTGCCCGCA GTTTTTATTA AACATAACGT GGGATCTCCA 6552
CGCGAATCTC GGGTACGTGT TCCGGACATG GGCTCTTCTC CGGTAGCGGC GGAGCTTCTA 6612
CATCCGAGCC CTGCTCCCAT CCCTCCAGCG ACTCATGGTC GCTCGGCAGC TCCTTGCTCC 6672
TAACAGTGGA GGCCAGACTT AGGCACAGCA CGATGCCCAC CACCACCAGT GTGCCGCACA 6732
AGGCCGTGGC GGTAGGGTAT GTGTCTGAAA ATGAGCTCGG GGAGCGGGCT TGCACCGCTG 6792
ACGCATTTGG AAGACTTAAG GCAGCGGCAG AAGAAGATGC AGGCAGCTGA GTTGTTGTGT 6852
TCTGATAAGA GTCAGAGGTA ACTCCCGTTG CGGTGCTGTT AACGGTGGAG GGCAGTGTAG 6912
TCTGAGCAGT ACTCGTTGCT GCCGCGCGCG CCACCAGACA TAATAGCTGA CAGACTAACA 6972
GACTGTTCCT TTCCATGGGT CTTTTCTGCA GTCACCGTCC TTGACACGAA GCTTGGGCTG 7032
CAGGTCGATC GACTCTAGAG GATCGATCCC CGGGCGAGCT C 7073

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 219 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val
  1               5                  10                  15

Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn
             20                  25                  30

Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly
         35                  40                  45

Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr
     50                  55                  60

Asp Pro Lys Phe Gln Gly Arg Phe Ile Ile Ser Ala Asp Asn Ser Lys
 65                  70                  75                  80

Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala
                 85                  90                  95

Val Tyr Phe Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
        115                 120                 125

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
    130                 135                 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145                 150                 155                 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                165                 170                 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
            180                 185                 190

Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His
        195                 200                 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val
    210                 215
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro
  1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
  1               5                  10                  15

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
             20                  25                  30

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
         35                  40                  45

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
     50                  55                  60

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Met His Gln
 65                  70                  75                  80

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
                 85                  90                  95
```

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
100 105

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
1 5 10 15

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
20 25 30

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
35 40 45

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
50 55 60

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
65 70 75 80

Asn Val Phe Ser Val Ser Val Met His Glu Ala Leu His Asn His Tyr
85 90 95

Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
100 105

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7864 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 9..711

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
AATTCACCAT GGGTGTGCCA ACTCAGGTAT TAGGATTACT GCTGCTGTGG CTTACAGATG     60
CAAGATGTGA TATCCAAATG ACACAATCTC CTTCTTCTCT AAGTGCTTCT GTCGGAGATA    120
GAGTAACAAT TACATGTAAG GCGAGTCAGG ACATTAGAAA GTATTTAAAC TGGTATCAGC    180
AAAAACCTGG GAAGGCTCCT AAGCTACTGA TTTATTATGC AACAAGTTTG GCAGATGGAG    240
TACCTTCTAG ATTTTCTGGT TCTGGCTCTG GAACAGACTA CACATTCACA ATTTCTTCTC    300
TCCAACCTGA GGACATTGCT ACATACTACT GCCTACAACA TGGTGAGAGT CCGTATACAT    360
TTGGACAAGG AACAAAACTA GAGATCACAA GAACTGTTGC GGCGCCGTCT GTCTTCATCT    420
TCCCGCCATC TGATGAGCAG TTGAAATCTG GAACTGCCTC TGTTGTGTGC CTGCTGAATA    480
ACTTCTATCC CAGAGAGGCC AAAGTACAGT GGAAGGTGGA TAACGCCCTC CAATCGGGTA    540
ACTCCCAGGA GAGTGTCACA GAGCAGGACA GCAAGGACAG CACCTACAGC CTCAGCAGCA    600
CCCTGACGCT GAGCAAAGCA GACTACGAGA AACACAAAGT CTACGCCTGC GAAGTCACCC    660
ATCAGGGCCT GAGCTCGCCC GTCACAAAGA GCTTCAACAG GGGAGAGTGT TAGAGGGAGA    720
AGTGCCCCCA CCTGCTCCTC AGTTCCAGCC TGGGGATCAT AATCAGCCAT ACCACATTTG    780
TAGAGGTTTT ACTTGCTTTA AAAAACCTCC CACACCTCCC CCTGAACCTG AAACATAAAA    840
TGAATGCAAT TGTTGTTGTT AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA    900
ATAGCATCAC AAATTTCACA AATAAAGCAT TTTTTTCACT GCATTCTAGT TGTGGTTTGT    960
CCAAACTCAT CAATGTATCT TATCATGTCT GGATCCTCTA CGCCGGACGC ATCGTGGCCG   1020
GCATCACCGG CGCCACAGGT GCGGTTGCTG GCGCCTATAT CGCCGACATC ACCGATGGGG   1080
AAGATCGGGC TCGCCACTTC GGGCTCATGA GCGCTTGTTT CGGCGTGGGT ATGGTGGCAG   1140
```

GCCCGTGGCC GGGGGACTGT TGGGCGCCAT CTCCTTGCAT GCACCATTCC TTGCGGCGGC 1200
GGTGCTCAAC GGCCTCAACC TACTACTGGG CTGCTTCCTA ATGCAGGAGT CGCATAAGGG 1260
AGAGCGTCGA CCTCGGGCCG CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC CCCCTGACGA 1320
GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC CCGACAGGAC TATAAAGATA 1380
CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT GCGCTCTCCT GTTCCGACCC TGCCGCTTAC 1440
CGGATACCTG TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG CTTTCTCAAT GCTCACGCTG 1500
TAGGTATCTC AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC 1560
CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA ACCCGGTAAG 1620
ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT 1680
AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT 1740
ATTTGGTATC TGCGCTCTGC TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG 1800
ATCCGGCAAA CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC 1860
GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA 1920
GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA TTATCAAAAA GGATCTTCAC 1980
CTAGATCCTT TTAAATTAAA AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAAAC 2040
TTGGTCTGAC AGTTACCAAT GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT 2100
TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT 2160
ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA CGCTCACCGG CTCCAGATTT 2220
ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA AGTGGTCCTG CAACTTTATC 2280
CGCCTCCATC CAGTCTATTA ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA 2340
TAGTTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG 2400
TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT 2460
GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC 2520
AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT 2580

AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG 2640
GCGACCGAGT TGCTCTTGCC CGGCGTCAAC ACGGGATAAT ACCGCGCCAC ATAGCAGAAC 2700
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TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAAGGG 2880
AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC CTTTTTCAAT ATTATTGAAG 2940
CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA 3000
ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCAT 3060
TATTATCATG ACATTAACCT ATAAAAATAG GCGTATCACG AGGCCCTGAT GGCTCTTTGC 3120
GGCACCCATC GTTCGTAATG TTCCGTGGCA CCGAGGACAA CCCTCAAGAG AAAATGTAAT 3180
CACACTGGCT CACCTTCGGG TGGGCCTTTC TGCGTTTATA AGGAGACACT TTATGTTTAA 3240
GAAGGTTGGT AAATTCCTTG CGGCTTTGGC AGCCAAGCTA GAGATCCGGC TGTGGAATGT 3300
GTGTCAGTTA GGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT 3360
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CCAGTTCCGC CCATTCTCCG CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG 3540
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CCGCACCCTG GACTGTGAGC CCAAGTGTGT AGAAGAGTTA CCTGAGTGGA ATTTTGATGG 3840
CTCTAGTACC TTTCAGTCTG AGGGCTCCAA CAGTGACATG TATCTCAGCC CTGTTGCCAT 3900
GTTTCGGGAC CCCTTCCGCA GAGATCCCAA CAAGCTGGTG TTCTGTGAAG TTTTCAAGTA 3960
CAACCGGAAG CCTGCAGAGA CCAATTTAAG GCACTCGTGT AAACGGATAA TGGACATGGT 4020

GAGCAACCAG CACCCCTGGT TTGGAATGGA ACAGGAGTAT ACTCTGATGG GAACAGATGG 4080
GCACCCTTTT GGTTGGCCTT CCAATGGCTT TCCTGGGCCC CAAGGTCCGT ATTACTGTGG 4140
TGTGGGCGCA GACAAAGCCT ATGGCAGGGA TATCGTGGAG GCTCACTACC GCGCCTGCTT 4200
GTATGCTGGG GTCAAGATTA CAGGAACAAA TGCTGAGGTC ATGCCTGCCC AGTGGGAACT 4260
CCAAATAGGA CCCTGTGAAG GAATCCGCAT GGGAGATCAT CTCTGGGTGG CCCGTTTCAT 4320
CTTNCATCGA GTATGTGAAG ACTTTGGGGT AATAGCAACC TTTGACCCCA AGCCCATTCC 4380
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GAATGGTCTG AAGCACATCG AGGAGGCCAT CGAGAAACTA AGCAAGCGGC ACCGGTACCA 4500
CATTCGAGCC TACGATCCCA AGGGGGGCCT GGACAATGCC CGTGGTCTGA CTGGGTTCCA 4560
CGAAACGTCC AACATCAACG ACTTTTCTGC TGGTGTCGCC AATCGCAGTG CCAGCATCCG 4620
CATTCCCCCG ACTGTCGGCC AGGAGAAGAA AGGTTACTTT GAAGACCGCG GCCCCTCTGC 4680
CAATTGTGAC CCCTTTGCAG TGACAGAAGC CATCGTCCGC ACATGCCTTC TCAATGAGAC 4740
TGGCCACGAG CCCTTCCAAT ACAAAAACTA ATTAGACTTT GAGTGATCTT GAGCCTTTCC 4800
TAGTTCATCC CACCCCGCCC CAGAGAGATC TTTGTGAAGG AACCTTACTT CTGTGGTGTG 4860
ACATAATTGG ACAAACTACC TACAGAGATT TAAAGCTCTA AGGTAAATAT AAAATTTTTA 4920
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GACCGATCCA GCCTCCGCGG CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG 6960
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TAGCTTAGCC TATAGGTGTG GGTTATTGAC CATTATTGAC CACTCCCCTA TTGGTGACGA 7140
TACTTTCCAT TACTAATCCA TAACATGGCT CTTTGCCACA ACTCTCTTTA TTGGCTATAT 7200
GCCAATACAC TGTCCTTCAG AGACTGACAC GGACTCTGTA TTTTTACAGG ATGGGGTCTC 7260
ATTTATTATT TACAAATTCA CATATACAAC ACCACCGTCC CCAGTGCCCG CAGTTTTTAT 7320
TAAACATAAC GTGGGATCTC CACGCGAATC TCGGGTACGT GTTCCGGACA TGGGCTCTTC 7380
TCCGGTAGCG GCGGAGCTTC TACATCCGAG CCCTGCTCCC ATGCCTCCAG CGACTCATGG 7440
TCGCTCGGCA TCTCCTTGCT CCTAACAGTG GAGGCCAGAC TTAGGCACAG CACGATGCCC 7500
ACCACCACCA GTGTGCCGCA CAAGGCCGTG GCGGTAGGGT ATGTGTCTGA AAATGAGCTC 7560
GGGGAGCGGG CTTGCACCGC TGACGCATTT GGAAGACTTA AGGCAGCGGC AGAAGAAGAT 7620
GCAGGCAGCT GAGTTGTTGT GTTCTGATAA GAGTCAGAGG TAACTCCCGT TGCGGTGCTG 7680
TTAACGGTGG AGGGCAGTGT AGTCTGAGCA GTACTCGTTG CTGCCGCGCG CGCCACCAGA 7740
CATAATAGCT GACAGACTAA CAGACTGTTC CTTTCCATGG GTCTTTTCTG CAGTCACCGT 7800
CCTTGACACG AAGCTTGGGC TGCAGGTCGA TCGACTCTAG AGGATCGATC CCCGGGCGAG 7860
CTCG 7864

WHAT IS CLAIMED IS:

1. A CDR-grafted antibody capable of inhibiting human tissue factor wherein the complementarity determining regions (CDRs) are derived from a non-human monoclonal antibody against tissue factor and the framework (FR) and constant (C) regions are derived from one or more human antibodies.

2. The CDR-grafted antibody of Claim 1 wherein said non-human monoclonal antibody is a murine antibody.

3. The CDR-grafted antibody of Claim 2 wherein said murine antibody is TF8-5G9.

4. The CDR-grafted antibody of Claim 1 wherein said CDRs of the heavy chain have the amino acid sequences:

CDR1	DDYMH	(SEQ ID NO:5)
CDR2	LIDPENGNTIYDPKFQG	(SEQ ID NO:6)
CDR3	DNSYYFDY	(SEQ ID NO:7)

and said CDRs of the light chain have the amino acid sequences:

CDR1	KASQDIRKYLN	(SEQ ID NO:8)
CDR2	YATSLAD	(SEQ ID NO:9)
CDR3	LQHGESPYT	(SEQ ID NO:10).

5. The CDR-grafted antibody of Claim 1 wherein the FR of the heavy chain is derived from the human antibody KOL.

6. The CDR-grafted antibody of Claim 1 wherein the FR of the light chain is derived from the human antibody REI.

7. The CDR-grafted antibody of Claim 1 wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:11.

8. The CDR-grafted antibody of Claim 1 or 7 wherein the light chain variable region has the amino acid sequence of SEQ ID NO:12.

9. The CDR-grafted antibody of Claim 1 wherein the heavy chain variable region has the amino acid sequence of SEQ ID NO:13.

10. The CDR-grafted antibody of Claim 1 or 9 wherein the light chain variable region has the amino acid sequence of SEQ ID NO:14.

11. The CDR-grafted antibody of Claim 1 wherein the heavy chain constant region is the human IgG4 constant region.

12. The CDR-grafted antibody of Claim 10 wherein the heavy chain constant region is the human IgG4 constant region.

13. The CDR-grafted antibody of Claim 1 wherein the light chain constant region is the human kappa constant region.

14. The CDR-grafted antibody of Claim 10 wherein the light chain constant region is the human kappa constant region.

15. CDR-grafted monoclonal antibody TF8HCDR1 x TF8LCDR1.

16. CDR-grafted monoclonal antibody TF8HCDR20 x TF8LCDR3.

17. A fragment of the CDR-grafted antibody of Claim 1 wherein said fragment is capable of inhibiting human tissue factor.

18. The fragment of Claim 17 wherein said fragment is an Fab or $F(ab')_2$ fragment.

19. A method of making the CDR-grafted antibody of Claim 1 comprising cotransfecting a host cell with an expression vector comprising a nucleic acid encoding the CDR-grafted antibody heavy chain and an expression vector comprising a nucleic acid encoding the CDR-grafted antibody light chain; culturing the transfected host cell; and recovering said CDR-grafted antibody.

20. A method of making the CDR-grafted antibody of Claim 1 comprising transfecting a host cell with an expression vector comprising a nucleic acid encoding the CDR-grafted antibody heavy chain and a nucleic acid encoding the CDR-grafted antibody light chain; culturing the transfected host cell; and recovering said CDR-grafted antibody.

21. The method of Claim 18 or 19 wherein said nucleic acid encoding the CDR-grafted antibody heavy chain has the sequence of nucleotides 1-2360 of SEQ ID NO:15.

22. The method of Claim 18 or 19 wherein said nucleic acid encoding the CDR-grafted light chain has the sequence of nucleotides 1-759 of SEQ ID NO:17.

23. The method of Claim 19 or 20 wherein said host cell is a bacterial cell, yeast cell, insect cell or mammalian cell.

24. The method of Claim 23 wherein said mammalian cell is a CHO cell, COS cell or myeloma cell.

25. The method of Claim 19 wherein said expression vector comprising a nucleic acid encoding the CDR-grafted antibody heavy chain is pEe6TF8HCDR20.

26. The method of Claim 19 wherein said expression vector comprising a nucleic acid encoding the CDR-grafted antibody light chain is pEe12TF8LCDR3.

27. A nucleic acid encoding the heavy chain of the CDR-grafted antibody of Claim 1.

28. A nucleic acid encoding the light chain of the CDR-grafted antibody of Claim 1.

29. The nucleic acid of Claim 27 having the sequence of nucleotides 1-2360 of SEQ ID NO:15.

30. The nucleic acid of Claim 28 having the sequence of nucleotides 1-759 of SEQ ID NO:17.

31. A method of attenuation of coagulation comprising administering a therapeutically effective amount of a CDR-grafted antibody capable of inhibiting human tissue factor to a patient in need of said attenuation.

32. The method of Claim 31 wherein said CDR-grafted antibody is TF8HCDR20 x TF84CDR3.

33. A method of treatment or prevention of thrombotic disorder comprising administering a therapeutically effective amount of a CDR-grafted antibody capable of inhibiting human tissue factor to a patient in need of said treatment or prevention.

34. The method of Claim 33 wherein said thrombotic disorder is intravascular coagulation, arterial restenosis or arteriosclerosis.

35. The method of Claim 33 or 34 wherein said CDR-grafted antibody is TF8HCDR20 x TF8LCDR3.

36. A pharmaceutical composition comprising at least one CDR-grafted antibody capable of inhibiting human tissue factor and a pharmaceutically acceptable carrier.

37. The pharmaceutical composition of Claim 36 wherein said CDR-grafted antibody is TF8HCDR20 x TF8LCDR3.

FIG. 1 A

Sequence of the murine TF8-5G9 heavy chain cDNA with protein translation. The essential regions of the cDNA are as follows:

Nucleotides	Region
1-10	5' untranslated region.
11-67	Start codon and leader sequence.
68-418	Variable region.
419-1390	Murine IgG1 constant region.
1391-1489	3' untranslated region.

Sequence Range: 1 to 1489

```
                10                  20                  30                  40
GGT CCT TAC A ATG AAA TGC AGC TGG GTC ATC TTC TTC CTG ATG GCA GTG
CCA GGA ATG T TAC TTT ACG TCG ACC CAG TAG AAG AAG GAC TAC CGT CAC
              Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val>

50                  60                  70                  80                  90
GTT ACA GGG GTC AAT TCA GAG ATT CAG CTG CAG CAG TCT GGG GCT GAG
CAA TGT CCC CAG TTA AGT CTC TAA GTC GAC GTC GTC AGA CCC CGA CTC
Val Thr Gly Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu>

100                 110                 120                 130                 140
CTT GTG AGG CCA GGG GCC TTA GTC AAG TTG TCC TGC AAA GCT TCT GGC
GAA CAC TCC GGT CCC CGG AAT CAG TTC AAC AGG ACG TTT CGA AGA CCG
Leu Val Arg Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly>

    150                 160                 170                 180                 190
TTC AAC ATT AAA GAC TAC TAT ATG CAC TGG GTG AAG CAG AGG CCT GAA
AAG TTG TAA TTT CTG ATG ATA TAC GTG ACC CAC TTC GTC TCC GGA CTT
Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu>

        200                 210                 220                 230                 240
CAG GGC CTG GAG TGG ATT GGA TTG ATT GAT CCT GAG AAT GGT AAT ACT
GTC CCG GAC CTC ACC TAA CCT AAC TAA CTA GGA CTC TTA CCA TTA TGA
Gln Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr>

            250                 260                 270                 280
ATA TAT GAC CCG AAG TTC CAG GGC AAG GCC AGT ATA ACA GCA GAC ACA
TAT ATA CTG GGC TTC AAG GTC CCG TTC CGG TCA TAT TGT CGT CTG TGT
Ile Tyr Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr>

290                 300                 310                 320                 330
TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT GAG GAC
AGG AGG TTG TGT CGG ATG GAC GTC GAG TCG TCG GAC TGT AGA CTC CTG
Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp>
```

FIG. 1 B

```
340                 350                 360                 370                 380
 *                   *                   *                   *                   *
ACT GCC GTC TAT TAC TGT GCT AGA GAT AAC TCG TAC TAC TTT GAC TAC
TGA CGG CAG ATA ATG ACA CGA TCT CTA TTG AGC ATG ATG AAA CTG ATG
Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr>

    390                 400                 410                 420                 430
     *                   *                   *                   *                   *
TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC AAA ACG ACA CCC
ACC CCG GTT CCG TGG TGA GAG TGT CAG AGG AGT CGG TTT TGC TGT GGG
Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro>

        440                 450                 460                 470                 480
         *                   *                   *                   *                   *
CCA TCT GTC TAT CCA CTG GCC CCT GGA TCT GCT GCC CAA ACT AAC TCC
GGT AGA CAG ATA GGT GAC CGG GGA CCT AGA CGA CGG GTT TGA TTG AGG
Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser>

            490                 500                 510                 520
             *                   *                   *                   *
ATG GTG ACC CTG GGA TGC CTG GTC AAG GGC TAT TTC CCT GAG CCA GTG
TAC CAC TGG GAC CCT ACG GAC CAG TTC CCG ATA AAG GGA CTC GGT CAC
Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val>

530                 540                 550                 560                 570
 *                   *                   *                   *                   *
ACA GTG ACC TGG AAC TCT GGA TCC CTG TCC AGC GGT GTG CAC ACC TTC
TGT CAC TGG ACC TTG AGA CCT AGG GAC AGG TCG CCA CAC GTG TGG AAG
Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe>

580                 590                 600                 610                 620
 *                   *                   *                   *                   *
CCA GCT GTC CTG CAG TCT GAC CTC TAC ACT CTG AGC AGC TCA GTG ACT
GGT CGA CAG GAC GTC AGA CTG GAG ATG TGA GAC TCG TCG AGT CAC TGA
Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr>

    630                 640                 650                 660                 670
     *                   *                   *                   *                   *
GTG CCC TCC AGC ACC TGG CCC AGC GAG ACC GTC ACC TGC AAC GTT GCC
CAC GGG AGG TCG TGG ACC GGG TCG CTC TGG CAG TGG ACG TTG CAA CGG
Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala>

        680                 690                 700                 710                 720
         *                   *                   *                   *                   *
CAC CCG GCC AGC AGC ACC AAG GTG GAC AAG AAA ATT GTG CCC AGG GAT
GTG GGC CGG TCG TCG TGG TTC CAC CTG TTC TTT TAA CAC GGG TCC CTA
His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp>

            730                 740                 750                 760
             *                   *                   *                   *
TGT GGT TGT AAG CCT TGC ATA TGT ACA GTC CCA GAA GTA TCA TCT GTC
ACA CCA ACA TTC GGA ACG TAT ACA TGT CAG GGT CTT CAT AGT AGA CAG
Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val>

770                 780                 790                 800                 810
 *                   *                   *                   *                   *
TTC ATC TTC CCC CCA AAG CCC AAG GAT GTG CTC ACC ATT ACT CTG ACT
AAG TAG AAG GGG GGT TTC GGG TTC CTA CAC GAG TGG TAA TGA GAC TGA
Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr>
```

FIG. 1 C

```
820          830          840          850          860
CCT AAG GTC ACG TGT GTT GTG GTA GAC ATC AGC AAG GAT GAT CCC GAG
GGA TTC CAG TGC ACA CAA CAC CAT CTG TAG TCG TTC CTA CTA GGG CTC
Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu>

    870          880          890          900          910
GTC CAG TTC AGC TGG TTT GTA GAT GAT GTG GAG GTG CAC ACA GCT CAG
CAG GTC AAG TCG ACC AAA CAT CTA CTA CAC CTC CAC GTG TGT CGA GTC
Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln>

        920          930          940          950          960
ACG CAA CCC CGG GAG GAG CAG TTC AAC AGC ACT TTC CGC TCA GTC AGT
TGC GTT GGG GCC CTC CTC GTC AAG TTG TCG TGA AAG GCG AGT CAG TCA
Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser>

            970          980          990         1000
GAA CTT CCC ATC ATG CAC CAG GAC TGG CTC AAT GGC AAG GAG TTC AAA
CTT GAA GGG TAG TAC GTG GTC CTG ACC GAG TTA CCG TTC CTC AAG TTT
Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys>

1010         1020         1030         1040         1050
TGC AGG GTC AAC AGT GCA GCT TTC CCT GCC CCC ATC GAG AAA ACC ATC
ACG TCC CAG TTG TCA CGT CGA AAG GGA CGG GGG TAG CTC TTT TGG TAG
Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile>

1060         1070         1080         1090         1100
TCC AAA ACC AAA GGC AGA CCG AAG GCT CCA CAG GTG TAC ACC ATT CCA
AGG TTT TGG TTT CCG TCT GGC TTC CGA GGT GTC CAC ATG TGG TAA GGT
Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro>

    1110         1120         1130         1140         1150
CCT CCC AAG GAG CAG ATG GCC AAG GAT AAA GTC AGT CTG ACC TGC ATG
GGA GGG TTC CTC GTC TAC CGG TTC CTA TTT CAG TCA GAC TGG ACG TAC
Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met>

        1160         1170         1180         1190         1200
ATA ACA GAC TTC TTC CCT GAA GAC ATT ACT GTG GAG TGG CAG TGG AAT
TAT TGT CTG AAG AAG GGA CTT CTG TAA TGA CAC CTC ACC GTC ACC TTA
Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn>

            1210         1220         1230         1240
GGG CAG CCA GCG GAG AAC TAC AAG AAC ACT CAG CCC ATC ATG GAC ACA
CCC GTC GGT CGC CTC TTG ATG TTC TTG TGA GTC GGG TAG TAC CTG TGT
Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr>

1250         1260         1270         1280         1290
GAT GGC TCT TAC TTC GTC TAC AGC AAG CTC AAT GTG CAG AAG AGC AAC
CTA CCG AGA ATG AAG CAG ATG TCG TTC GAG TTA CAC GTC TTC TCG TTG
Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn>
```

FIG. 1 D

```
1300        1310        1320        1330        1340
  *           *           *           *           *
TGG GAG GCA GGA AAT ACT TTC ACC TGC TCT GTG TTA CAT GAG GGC CTG
ACC CTC CGT CCT TTA TGA AAG TGG ACG AGA CAC AAT GTA CTC CCG GAC
Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu>

   1350        1360        1370        1380        1390
     *           *           *           *           *
CAC AAC CAC CAT ACT GAG AAG AGC CTC TCC CAC TCT CCT GGT AAA TG ATC
GTG TTG GTG GTA TGA CTC TTC TCG GAG AGG GTG AGA GGA CCA TTT AC TAG
His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys>

   1400        1410        1420        1430        1440
     *           *           *           *           *
CCA GTG TCC TTG GAG CCC TCT GGT CCT ACA GGA CTC TGA CAC CTA CCT
GGT CAC AGG AAC CTC GGG AGA CCA GGA TGT CCT GAG ACT GTG GAT GGA

      1450        1460        1470        1480
        *           *           *           *
CCA CCC CTC CCT GTA TAA ATA AAG CAC CCA GCA CTG CCT TGG ACC C
GGT GGG GAG GGA CAT ATT TAT TTC GTG GGT CGT GAC GGA ACC TGG G
```

Sequence of the murine TF8-5G9 light chain cDNA with protein translation. The essential regions of the cDNA are as follows:

FIG. 2 A

Nucleotides	Region
1-4	5' untranslated.
5-64	Start codon and leader sequence.
65-385	Variable region.
386-706	Murine kappa constant region.
707-917	3' untranslated region.
918-937	Poly A tail.

Sequence Range: 1 to 937

```
               10           20           30           40
GGA C ATG CGG GCC CCT GCT CAG TTT TTT GGG ATC TTG TTG CTC TGG TTT
CCT G TAC GCC CGG GGA CGA GTC AAA AAA CCC TAG AAC AAC GAG ACC AAA
      Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe>

50           60           70           80           90
CCA GGT ATC AGA TGT GAC ATC AAG ATG ACC CAG TCT CCA TCC TCC ATG
GGT CCA TAG TCT ACA CTG TAG TTC TAC TGG GTC AGA GGT AGG AGG TAC
Pro Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met>

100           110          120          130          140
TAT GCA TCG CTG GGA GAG AGA GTC ACT ATC ACT TGT AAG GCG AGT CAG
ATA CGT AGC GAC CCT CTC TCT CAG TGA TAG TGA ACA TTC CGC TCA GTC
Tyr Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln>

   150          160          170          180          190
GAC ATT AGA AAG TAT TTA AAC TGG TAC CAG CAG AAA CCA TGG AAA TCT
CTG TAA TCT TTC ATA AAT TTG ACC ATG GTC GTC TTT GGT ACC TTT AGA
Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser>

      200          210          220          230          240
CCT AAG ACC CTG ATC TAT TAT GCA ACA AGC TTG GCA GAT GGG GTC CCA
GGA TTC TGG GAC TAG ATA ATA CGT TGT TCG AAC CGT CTA CCC CAG GGT
Pro Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro>

         250          260          270          280
TCA AGA TTC AGT GGC AGT GGA TCT GGG CAA GAT TAT TCT CTA ACC ATC
AGT TCT AAG TCA CCG TCA CCT AGA CCC GTT CTA ATA AGA GAT TGG TAG
Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile>

290          300          310          320          330
AGC AGC CTG GAG TCT GAC GAT ACA GCA ACT TAT TAC TGT CTA CAA CAT
TCG TCG GAC CTC AGA CTG CTA TGT CGT TGA ATA ATG ACA GAT GTT GTA
Ser Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His>
```

FIG. 2B

```
340 350 360 370 380
GGT GAG AGC CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAC
CCA CTC TCG GGC ATG TGC AAG CCT CCC CCC TGG TTC GAC CTT TAT TTG
Gly Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn>

390 400 410 420 430
AGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA TCC AGT GAG
TCC CGA CTA CGA CGT GGT TGA CAT AGG TAG AAG GGT GGT AGG TCA CTC
Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu>

440 450 460 470 480
CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC TTC TTG AAC AAC TTC
GTC AAT TGT AGA CCT CCA CGG AGT CAG CAC ACG AAG AAC TTG TTG AAG
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe>

490 500 510 520
TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT GAT GGC AGT GAA CGA
ATG GGG TTT CTG TAG TTA CAG TTC ACC TTC TAA CTA CCG TCA CTT GCT
Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg>

530 540 550 560 570
CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG GAC AGC AAA GAC AGC
GTT TTA CCG CAG GAC TTG TCA ACC TGA CTA GTC CTG TCG TTT CTG TCG
Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser>

580 590 600 610 620
ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC AAG GAC GAG TAT GAA
TGG ATG TCG TAC TCG TCG TGG GAG TGC AAC TGG TTC CTG CTC ATA CTT
Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu>

630 640 650 660 670
CGA CAT AAC AGC TAT ACC TGT GAG GCC ACT CAC AAG ACA TCA ACT TCA
GCT GTA TTG TCG ATA TGG ACA CTC CGG TGA GTG TTC TGT AGT TGA AGT
Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser>

680 690 700 710 720
CCC ATT GTC AAG AGC TTC AAC AGG AAT GAG TGT TA GAG ACA AAG GTC CTG
GGG TAA CAG TTC TCG AAG TTG TCC TTA CTC ACA AT CTC TGT TTC CAG GAC
Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys>

730 740 750 760 770
AGA CGC CAC CAC CAG CTC CCC AGC TCC ATC CTA TCT TCC CTT CTA AGG
TCT GCG GTG GTG GTC GAG GGG TCG AGG TAG GAT AGA AGG GAA GAT TCC

780 790 800 810
TCT TGG AGG CTT CCC CAC AAG CGA CCT ACC ACT GTT GCG GTG CTC CAA
AGA ACC TCC GAA GGG GTG TTC GCT GGA TGG TGA CAA CGC CAC GAG GTT
```

FIG. 2 C

```
820          830          840          850          860
 *            *            *            *            *
ACC TCC TCC CCA CCT CCT TCT CCT CCT CCT CCC TTT CCT TGG CTT TTA
TGG AGG AGG GGT GGA GGA AGA GGA GGA GGA GGG AAA GGA ACC GAA AAT

870          880          890          900          910
 *            *            *            *            *
TCA TGC TAA TAT TTG CAG AAA ATA TTC AAT AAA GTG AGT CTT TGC ACT
AGT ACG ATT ATA AAC GTC TTT TAT AAG TTA TTT CAC TCA GAA ACG TGA

    920          930
     *            *
TGA AAA AAA AAA AAA AAA AAA A
ACT TTT TTT TTT TTT TTT TTT T
```

FIG. 3

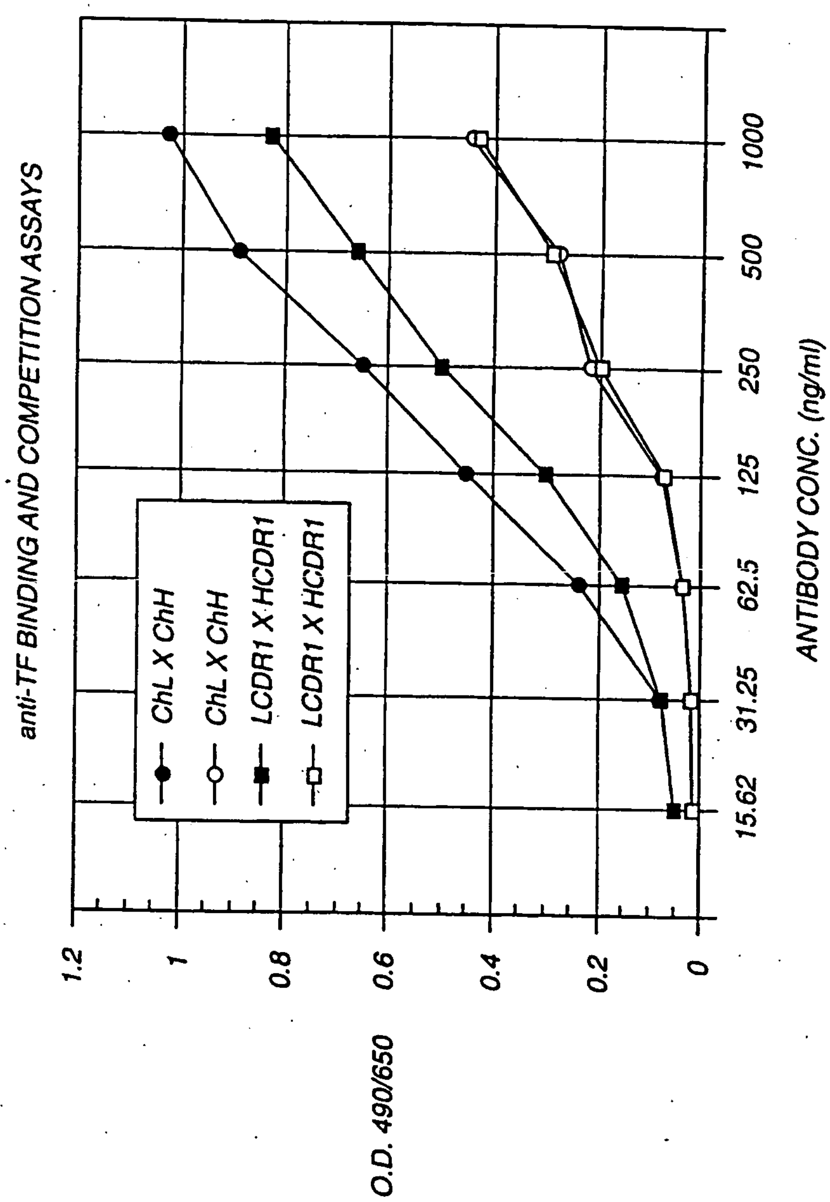

anti-TF BINDING AND COMPETITION ASSAYS
ChL X ChH
ChL X ChH
LCDR1 X HCDR1
LCDR1 X HCDR1
O.D. 490/650
1.2
1
0.8
0.6
0.4
0.2
0
15.62
31.25
62.5
125
250
500
1000
ANTIBODY CONC. (ng/ml)

FIG. 4 A The pEe6TF8HCDR20 expression vector DNA sequence. The coding regions of the TF8-5G9 CDR-grafted HC gene, TF8HCDR20, are translated.

Sequence Range: 1 to 7073

```
                 10          20          30          40
                  *           *           *           *
GAA TTC GCC GCC ACC ATG GAA TGG AGC TGG GTC TTT CTC TTC TTC TTG
CTT AAG CGG CGG TGG TAC CTT ACC TCG ACC CAG AAA GAG AAG AAG AAC
                    Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu>

50          60          70          80          90
 *           *           *           *           *
TCA GTA ACT ACA GGT GTA CAC TCA CAA GTT CAG CTG GTG GAG TCT GGA
AGT CAT TGA TGT CCA CAT GTG AGT GTT CAA GTC GAC CAC CTC AGA CCT
Ser Val Thr Thr Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly>

   100         110         120         130         140
    *           *           *           *           *
GGA GGA GTA GTA CAA CCT GGA AGG TCA CTG AGA CTG TCT TGT AAG GCT
CCT CCT CAT CAT GTT GGA CCT TCC AGT GAC TCT GAC AGA ACA TTC CGA
Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala>

      150         160         170         180         190
       *           *           *           *           *
AGT GGA TTC AAT ATC AAG GAC TAT TAT ATG CAC TGG GTC AGA CAA GCT
TCA CCT AAG TTA TAG TTC CTG ATA ATA TAC GTG ACC CAG TCT GTT CGA
Ser Gly Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala>

         200         210         220         230         240
          *           *           *           *           *
CCT GGA AAA GGA CTC GAG TGG ATA GGT TTA ATT GAT CCT GAG AAT GGT
GGA CCT TTT CCT GAG CTC ACC TAT CCA AAT TAA CTA GGA CTC TTA CCA
Pro Gly Lys Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly>

            250         260         270         280
             *           *           *           *
AAC ACG ATA TAT GAT CCC AAG TTC CAA GGA AGA TTC ACA ATT TCT GCA
TTG TGC TAT ATA CTA GGG TTC AAG GTT CCT TCT AAG TGT TAA AGA CGT
Asn Thr Ile Tyr Asp Pro Lys Phe Gln Gly Arg Phe Thr Ile Ser Ala>

290         300         310         320         330
 *           *           *           *           *
GAC AAC TCT AAG AAT ACA CTG TTC CTG CAG ATG GAC TCA CTC AGA CCT
CTG TTG AGA TTC TTA TGT GAC AAG GAC GTC TAC CTG AGT GAG TCT GGA
Asp Asn Ser Lys Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro>

   340         350         360         370         380
    *           *           *           *           *
GAG GAT ACA GCA GTC TAC TAT TGT GCT AGA GAT AAC AGT TAT TAC TTC
CTC CTA TGT CGT CAG ATG ATA ACA CGA TCT CTA TTG TCA ATA ATG AAG
Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe>

      390         400         410         420         430
       *           *           *           *           *
GAC TAC TGG GGC CAA GGA ACA CCA GTC ACC GTG AGC TCA GCT TCC ACC
CTG ATG ACC CCG GTT CCT TGT GGT CAG TGG CAC TCG AGT CGA AGG TGG
Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr>
```

FIG. 4 B

```
        440         450         460         470         480
         *           *           *           *           *
AAG GGC CCA TCC GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC
TTC CCG GGT AGG CAG AAG GGG GAC CGC GGG ACG AGG TCC TCG TGG AGG
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser>

          490         500         510         520
           *           *           *           *
GAG AGC ACA GCC GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA
CTC TCG TGT CGG CGG GAC CCG ACG GAC CAG TTC CTG ATG AAG GGG CTT
Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu>

530         540         550         560         570
 *           *           *           *           *
CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC
GGC CAC TGC CAC AGC ACC TTG AGT CCG CGG GAC TGG TCG CCG CAC GTG
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His>

    580         590         600         610         620
     *           *           *           *           *
ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC
TGG AAG GGC CGA CAG GAT GTC AGG AGT CCT GAG ATG AGG GAG TCG TCG
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser>

      630         640         650         660         670
       *           *           *           *           *
GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC
CAC CAC TGG CAC GGG AGG TCG TCG AAC CCG TGC TTC TGG ATG TGG ACG
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys>

        680         690         700         710         720
         *           *           *           *           *
AAC GTA GAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GGT
TTG CAT CTA GTG TTC GGG TCG TTG TGG TTC CAC CTG TTC TCT CAA CCA
Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val>

          730         740         750         760
           *           *           *           *
GAG AGG CCA GCA CAG GGC AGG GAG GGT GTC TGC TGG AAG CCA GGC TCA
CTC TCC GGT CGT GTC CCG TCC CTC CCA CAG ACG ACC TTC GGT CCG AGT

770         780         790         800         810
 *           *           *           *           *
GCC CTC CTG CCT GGA CGC ACC CCG GCT GTG CAG CCC CAG CCC AGG GCA
CGG GAG GAC GGA CCT GCG TGG GGC CGA CAC GTC GGG GTC GGG TCC CGT

    820         830         840         850         860
     *           *           *           *           *
GCA AGG CAT GCC CCA TCT GTC TCC TCA CCC GGA GGC CTC TGA CCA CCC
CGT TCC GTA CGG GGT AGA CAG AGG AGT GGG CCT CCG GAG ACT GGT GGG

      870         880         890         900         910
       *           *           *           *           *
CAC TCA TGC TCA GGG AGA GGG TCT TCT GGA TTT TTC CAC CAG GCT CCG
GTG AGT ACG AGT CCC TCT CCC AGA AGA CCT AAA AAG GTG GTC CGA GGC
```

FIG. 4 C

```
         920            930            940            950            960
GGC AGC CAC AGG CTG GAT GCC CCT ACC CCA GGC CCT GCG CAT ACA GGG
CCG TCG GTG TCC GAC CTA CGG GGA TGG GGT CCG GGA CGC GTA TGT CCC

            970            980            990           1000
GCA GGT GCT GCG CTC AGA CCT GCC AAG AGC CAT ATC CGG GAG GAC CCT
CGT CCA CGA CGC GAG TCT GGA CGG TTC TCG GTA TAG GCC CTC CTG GGA

1010          1020           1030           1040           1050
GCC CCT GAC CTA AGC CCA CCC CAA AGG CCA AAC TCT CCA CTC CCT CAG
CGG GGA CTG GAT TCG GGT GGG GTT TCC GGT TTG AGA GGT GAG GGA GTC

   1060           1070           1080           1090           1100
CTC AGA CAC CTT CTC TCC TCC CAG ATT CGA GTA ACT CCC AAT CTT CTC
GAG TCT GTG GAA GAG AGG AGG GTC TAA GCT CAT TGA GGG TTA GAA GAG

     1110           1120           1130           1140           1150
TCT GCA GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA GGT AAG
AGA CGT CTC AGG TTT ATA CCA GGG GGT ACG GGT AGT ACG GGT CCA TTC
        Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro>

        1160           1170           1180           1190           1200
CCA ACC CAG GCC TCG CCC TCC AGC TCA AGG CGG GAC AGG TGC CCT AGA
GGT TGG GTC CGG AGC GGG AGG TCG AGT TCC GCC CTG TCC ACG GGA TCT

           1210           1220           1230           1240
GTA GCC TGC ATC CAG GGA CAG GCC CCA GCC GGG TGC TGA CGC ATC CAC
CAT CGG ACG TAG GTC CCT GTC CGG GGT CGG CCC ACG ACT GCG TAG GTG

1250          1260           1270           1280           1290
CTC CAT CTC TTC CTC AGC A CCT GAG TTC CTG GGG GGA CCA TCA GTC TTC
GAG GTA GAG AAG GAG TCG T GGA CTC AAG GAC CCC CCT GGT AGT CAG AAG
                          Pro Glu Phe Leu Gly Gly Pro Ser Val Phe>

 1300           1310           1320           1330           1340
CTG TTC CCC CCA AAA CCC AAG GAC ACT CTC ATG ATC TCC CGG ACC CCT
GAC AAG GGG GGT TTT GGG TTC CTG TGA GAG TAC TAG AGG GCC TGG GGA
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro>

    1350           1360           1370           1380           1390
GAG GTC ACG TGC GTG GTG GTG GAC GTG AGC CAG GAA GAC CCC GAG GTC
CTC CAG TGC ACG CAC CAC CAC CTG CAC TCG GTC CTT CTG GGG CTC CAG
Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val>

       1400           1410           1420           1430           1440
CAG TTC AAC TGG TAC GTG GAT GGC GTG GAG GTG CAT AAT GCC AAG ACA
GTC AAG TTG ACC ATG CAC CTA CCG CAC CTC CAC GTA TTA CGG TTC TGT
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr>
```

FIG. 4 D

```
        1450            1460            1470            1480
          *               *               *               *
AAG CCG CGG GAG GAG CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC
TTC GGC GCC CTC CTC GTC AAG TTG TCG TGC ATG GCA CAC CAG TCG CAG
Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val>

1490            1500            1510            1520            1530
  *               *               *               *               *
CTC ACC GTC CTG CAC CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC
GAG TGG CAG GAC GTG GTC CTG ACC GAC TTG CCG TTC CTC ATG TTC ACG
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys>

 1540            1550            1560            1570            1580
   *               *               *               *               *
AAG GTC TCC AAC AAA GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC
TTC CAG AGG TTG TTT CCG GAG GGC AGG AGG TAG CTC TTT TGG TAG AGG
Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser>

   1590            1600            1610            1620            1630
     *               *               *               *               *
AAA GCC AAA GG TGG GAC CCA CGG GGT GCG AGG GCC ACA TGG ACA GAG GTC
TTT CGG TTT CC ACC CTG GGT GCC CCA CGC TCC CGG TGT ACC TGT CTC CAG
Lys Ala Lys>

   1640            1650            1660            1670            1680
     *               *               *               *               *
AGC TCG GCC CAC CCT CTG CCC TGG GAG TGA CCG CTG TGC CAA CCT CTG
TCG AGC CGG GTG GGA GAC GGG ACC CTC ACT GGC GAC ACG GTT GGA GAC

      1690            1700            1710            1720            1730
        *               *               *               *               *
TCC CTA CA GGG CAG CCC CGA GAG CCA CAG GTG TAC ACC CTG CCC CCA TCC
AGG GAT GT CCC GTC GGG GCT CTC GGT GTC CAC ATG TGG GAC GGG GGT AGG
           Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser>

      1740            1750            1760            1770            1780
        *               *               *               *               *
CAG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA
GTC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT
Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys>

        1790            1800            1810            1820
          *               *               *               *
GGC TTC TAC CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG
CCG AAG ATG GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln>

1830            1840            1850            1860            1870
  *               *               *               *               *
CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC
GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly>

 1880            1890            1900            1910            1920
   *               *               *               *               *
TCC TTC TTC CTC TAC AGC AGG CTA ACC GTG GAC AAG AGC AGG TGG CAG
AGG AAG AAG GAG ATG TCG TCC GAT TGG CAC CTG TTC TCG TCC ACC GTC
Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln>
```

FIG. 4 E

```
1930 1940 1950 1960 1970
GAG GGG AAT GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC
CTC CCC TTA CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG
Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn>

1980 1990 2000 2010 2020
CAC TAC ACA CAG AAG AGC CTC TCC CTG TCT CTG GGT AAA T GAG TGC CAG
GTG ATG TGT GTC TTC TCG GAG AGG GAC AGA GAC CCA TTT A CTC ACG GTC
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Xxx>

2030 2040 2050 2060 2070
GGC CGG CAA GCC CCC GCT CCC CGG GCT CTC GGG GTC GCG CGA GGA TGC
CCG GCC GTT CGG GGG CGA GGG GCC CGA GAG CCC CAG CGC GCT CCT ACG

2080 2090 2100 2110
TTG GCA CGT ACC CCG TCT ACA TAC TTC CCA GGC ACC CAG CAT GGA AAT
AAC CGT GCA TGG GGC AGA TGT ATG AAG GGT CCG TGG GTC GTA CCT TTA

2120 2130 2140 2150 2160
AAA GCA CCC ACC ACT GCC CTG GGC CCC TGT GAG ACT GTG ATG GTT CTT
TTT CGT GGG TGG TGA CGG GAC CCG GGG ACA CTC TGA CAC TAC CAA GAA

2170 2180 2190 2200 2210
TCC ACG GGT CAG GCC GAG TCT GAG GCC TGA GTG ACA TGA GGG AGG CAG
AGG TGC CCA GTC CGG CTC AGA CTC CGG ACT CAC TGT ACT CCC TCC GTC

2220 2230 2240 2250 2260
AGC GGG TCC CAC TGT CCC CAC ACT GGC CCA GGC TGT GCA GGT GTG CCT
TCG CCC AGG GTG ACA GGG GTG TGA CCG GGT CCG ACA CGT CCA CAC GGA

2270 2280 2290 2300 2310
GGG CCA CCT AGG GTG GGG CTC AGC CAG GGG CTG CCC TCG GCA GGG TGG
CCC GGT GGA TCC CAC CCC GAG TCG GTC CCC GAC GGG AGC CGT CCC ACC

2320 2330 2340 2350
GGG ATT TGC CAG CGT GGC CCT CCC TCC AGC AGC AGG ACT CTA GAG GAT
CCC TAA ACG GTC GCA CCG GGA GGG AGG TCG TCG TCC TGA GAT CTC CTA

2360 2370 2380 2390 2400
CAT AAT CAG CCA TAC CAC ATT TGT AGA GGT TTT ACT TGC TTT AAA AAA
GTA TTA GTC GGT ATG GTG TAA ACA TCT CCA AAA TGA ACG AAA TTT TTT

2410 2420 2430 2440 2450
CCT CCC ACA CCT CCC CCT GAA CCT GAA ACA TAA AAT GAA TGC AAT TGT
GGA GGG TGT GGA GGG GGA CTT GGA CTT TGT ATT TTA CTT ACG TTA ACA
```

FIG. 4 F

```
    2460      2470      2480      2490      2500
TGT TGT TAA CTT GTT TAT TGC AGC TTA TAA TGG TTA CAA ATA AAG CAA
ACA ACA ATT GAA CAA ATA ACG TCG AAT ATT ACC AAT GTT TAT TTC GTT

    2510      2520      2530      2540      2550
TAG CAT CAC AAA TTT CAC AAA TAA AGC ATT TTT TTC ACT GCA TTC TAG
ATC GTA GTG TTT AAA GTG TTT ATT TCG TAA AAA AAG TGA CGT AAG ATC

    2560      2570      2580      2590
TTG TGG TTT GTC CAA ACT CAT CAA TGT ATC TTA TCA TGT CTG GAT CCT
AAC ACC AAA CAG GTT TGA GTA GTT ACA TAG AAT AGT ACA GAC CTA GGA

2600      2610      2620      2630      2640
CTA CGC CGG ACG CAT CGT GGC CGG CAT CAC CGG CGC CAC AGG TGC GGT
GAT GCG GCC TGC GTA GCA CCG GCC GTA GTG GCC GCG GTG TCC ACG CCA

    2650      2660      2670      2680      2690
TGC TGG CGC CTA TAT CGC CGA CAT CAC CGA TGG GGA AGA TCG GGC TCG
ACG ACC GCG GAT ATA GCG GCT GTA GTG GCT ACC CCT TCT AGC CCG AGC

    2700      2710      2720      2730      2740
CCA CTT CGG GCT CAT GAG CGC TTG TTT CGG CGT GGG TAT GGT GGC AGG
GGT GAA GCC CGA GTA CTC GCG AAC AAA GCC GCA CCC ATA CCA CCG TCC

    2750      2760      2770      2780      2790
CCC GTG GCC GGG GGA CTG TTG GGC GCC ATC TCC TTG CAT GCA CCA TTC
GGG CAC CGG CCC CCT GAC AAC CCG CGG TAG AGG AAC GTA CGT GGT AAG

    2800      2810      2820      2830
CTT GCG GCG GCG GTG CTC AAC GGC CTC AAC CTA CTA CTG GGC TGC TTC
GAA CGC CGC CGC CAC GAG TTG CCG GAG TTG GAT GAT GAC CCG ACG AAG

2840      2850      2860      2870      2880
CTA ATG CAG GAG TCG CAT AAG GGA GAG CGT CGA CCT CGG GCC GCG TTG
GAT TAC GTC CTC AGC GTA TTC CCT CTC GCA GCT GGA GCC CGG CGC AAC

    2890      2900      2910      2920      2930
CTG GCG TTT TTC CAT AGG CTC CGC CCC CCT GAC GAG CAT CAC AAA AAT
GAC CGC AAA AAG GTA TCC GAG GCG GGG GGA CTG CTC GTA GTG TTT TTA

    2940      2950      2960      2970      2980
CGA CGC TCA AGT CAG AGG TGG CGA AAC CCG ACA GGA CTA TAA AGA TAC
GCT GCG AGT TCA GTC TCC ACC GCT TTG GGC TGT CCT GAT ATT TCT ATG
```

FIG. 4 G

```
        2990           3000            3010           3020           3030
          *              *               *              *              *
CAG GCG TTT CCC CCT GGA AGC TCC CTC GTG CGC TCT CCT GTT CCG ACC
GTC CGC AAA GGG GGA CCT TCG AGG GAG CAC GCG AGA GGA CAA GGC TGG

           3040           3050           3060           3070
             *              *              *              *
CTG CCG CTT ACC GGA TAC CTG TCC GCC TTT CTC CCT TCG GGA AGC GTG
GAC GGC GAA TGG CCT ATG GAC AGG CGG AAA GAG GGA AGC CCT TCG CAC

3080           3090            3100           3110           3120
  *              *               *              *              *
GCG CTT TCT CAA TGC TCA CGC TGT AGG TAT CTC AGT TCG GTG TAG GTC
CGC GAA AGA GTT ACG AGT GCG ACA TCC ATA GAG TCA AGC CAC ATC CAG

 3130           3140           3150           3160           3170
   *              *              *              *              *
GTT CGC TCC AAG CTG GGC TGT GTG CAC GAA CCC CCC GTT CAG CCC GAC
CAA GCG AGG TTC GAC CCG ACA CAC GTG CTT GGG GGG CAA GTC GGG CTG

    3180           3190           3200           3210           3220
      *              *              *              *              *
CGC TGC GCC TTA TCC GGT AAC TAT CGT CTT GAG TCC AAC CCG GTA AGA
GCG ACG CGG AAT AGG CCA TTG ATA GCA GAA CTC AGG TTG GGC CAT TCT

       3230           3240           3250           3260           3270
         *              *              *              *              *
CAC GAC TTA TCG CCA CTG GCA GCA GCC ACT GGT AAC AGG ATT AGC AGA
GTG CTG AAT AGC GGT GAC CGT CGT CGG TGA CCA TTG TCC TAA TCG TCT

          3280           3290           3300           3310
            *              *              *              *
GCG AGG TAT GTA GGC GGT GCT ACA GAG TTC TTG AAG TGG TGG CCT AAC
CGC TCC ATA CAT CCG CCA CGA TGT CTC AAG AAC TTC ACC ACC GGA TTG

3320           3330           3340           3350           3360
  *              *              *              *              *
TAC GGC TAC ACT AGA AGG ACA GTA TTT GGT ATC TGC GCT CTG CTG AAG
ATG CCG ATG TGA TCT TCC TGT CAT AAA CCA TAG ACG CGA GAC GAC TTC

 3370           3380           3390           3400           3410
   *              *              *              *              *
CCA GTT ACC TTC GGA AAA AGA GTT GGT AGC TCT TGA TCC GGC AAA CAA
GGT CAA TGG AAG CCT TTT TCT CAA CCA TCG AGA ACT AGG CCG TTT GTT

    3420           3430           3440           3450           3460
      *              *              *              *              *
ACC ACC GCT GGT AGC GGT GGT TTT TTT GTT TGC AAG CAG CAG ATT ACG
TGG TGG CGA CCA TCG CCA CCA AAA AAA CAA ACG TTC GTC GTC TAA TGC

       3470           3480           3490           3500           3510
         *              *              *              *              *
CGC AGA AAA AAA GGA TCT CAA GAA GAT CCT TTG ATC TTT TCT ACG GGG
GCG TCT TTT TTT CCT AGA GTT CTT CTA GGA AAC TAG AAA AGA TGC CCC
```

FIG. 4 H

```
      3520        3530        3540        3550
TCT GAC GCT CAG TGG AAC GAA AAC TCA CGT TAA GGG ATT TTG GTC ATG
AGA CTG CGA GTC ACC TTG CTT TTG AGT GCA ATT CCC TAA AAC CAG TAC

3560        3570        3580        3590        3600
AGA TTA TCA AAA AGG ATC TTC ACC TAG ATC CTT TTA AAT TAA AAA TGA
TCT AAT AGT TTT TCC TAG AAG TGG ATC TAG GAA AAT TTA ATT TTT ACT

  3610        3620        3630        3640        3650
AGT TTT AAA TCA ATC TAA AGT ATA TAT GAG TAA ACT TGG TCT GAC AGT
TCA AAA TTT AGT TAG ATT TCA TAT ATA CTC ATT TGA ACC AGA CTG TCA

   3660        3670        3680        3690        3700
TAC CAA TGC TTA ATC AGT GAG GCA CCT ATC TCA GCG ATC TGT CTA TTT
ATG GTT ACG AAT TAG TCA CTC CGT GGA TAG AGT CGC TAG ACA GAT AAA

    3710        3720        3730        3740        3750
CGT TCA TCC ATA GTT GCC TGA CTC CCC GTC GTG TAG ATA ACT ACG ATA
GCA AGT AGG TAT CAA CGG ACT GAG GGG CAG CAC ATC TAT TGA TGC TAT

      3760        3770        3780        3790
CGG GAG GGC TTA CCA TCT GGC CCC AGT GCT GCA ATG ATA CCG CGA GAC
GCC CTC CCG AAT GGT AGA CCG GGG TCA CGA CGT TAC TAT GGC GCT CTG

3800        3810        3820        3830        3840
CCA CGC TCA CCG GCT CCA GAT TTA TCA GCA ATA AAC CAG CCA GCC GGA
GGT GCG AGT GGC CGA GGT CTA AAT AGT CGT TAT TTG GTC GGT CGG CCT

  3850        3860        3870        3880        3890
AGG GCC GAG CGC AGA AGT GGT CCT GCA ACT TTA TCC GCC TCC ATC CAG
TCC CGG CTC GCG TCT TCA CCA GGA CGT TGA AAT AGG CGG AGG TAG GTC

   3900        3910        3920        3930        3940
TCT ATT AAT TGT TGC CGG GAA GCT AGA GTA AGT AGT TCG CCA GTT AAT
AGA TAA TTA ACA ACG GCC CTT CGA TCT CAT TCA TCA AGC GGT CAA TTA

    3950        3960        3970        3980        3990
AGT TTG CGC AAC GTT GTT GCC ATT GCT ACA GGC ATC GTG GTG TCA CGC
TCA AAC GCG TTG CAA CAA CGG TAA CGA TGT CCG TAG CAC CAC AGT GCG

      4000        4010        4020        4030
TCG TCG TTT GGT ATG GCT TCA TTC AGC TCC GGT TCC CAA CGA TCA AGG
AGC AGC AAA CCA TAC CGA AGT AAG TCG AGG CCA AGG GTT GCT AGT TCC
```

FIG. 4 I

```
4040          4050          4060          4070          4080
CGA GTT ACA TGA TCC CCC ATG TTG TGC AAA AAA GCG GTT AGC TCC TTC
GCT CAA TGT ACT AGG GGG TAC AAC ACG TTT TTT CGC CAA TCG AGG AAG

   4090          4100          4110          4120          4130
GGT CCT CCG ATC GTT GTC AGA AGT AAG TTG GCC GCA GTG TTA TCA CTC
CCA GGA GGC TAG CAA CAG TCT TCA TTC AAC CGG CGT CAC AAT AGT GAG

      4140          4150          4160          4170          4180
ATG GTT ATG GCA GCA CTG CAT AAT TCT CTT ACT GTC ATG CCA TCC GTA
TAC CAA TAC CGT CGT GAC GTA TTA AGA GAA TGA CAG TAC GGT AGG CAT

         4190          4200          4210          4220          4230
AGA TGC TTT TCT GTG ACT GGT GAG TAC TCA ACC AAG TCA TTC TGA GAA
TCT ACG AAA AGA CAC TGA CCA CTC ATG AGT TGG TTC AGT AAG ACT CTT

             4240          4250          4260          4270
TAG TGT ATG CGG CGA CCG AGT TGC TCT TGC CCG GCG TCA ACA CGG GAT
ATC ACA TAC GCC GCT GGC TCA ACG AGA ACG GGC CGC AGT TGT GCC CTA

4280          4290          4300          4310          4320
AAT ACC GCG CCA CAT AGC AGA ACT TTA AAA GTG CTC ATC ATT GGA AAA
TTA TGG CGC GGT GTA TCG TCT TGA AAT TTT CAC GAG TAG TAA CCT TTT

   4330          4340          4350          4360          4370
CGT TCT TCG GGG CGA AAA CTC TCA AGG ATC TTA CCG CTG TTG AGA TCC
GCA AGA AGC CCC GCT TTT GAG AGT TCC TAG AAT GGC GAC AAC TCT AGG

      4380          4390          4400          4410          4420
AGT TCG ATG TAA CCC ACT CGT GCA CCC AAC TGA TCT TCA GCA TCT TTT
TCA AGC TAC ATT GGG TGA GCA CGT GGG TTG ACT AGA AGT CGT AGA AAA

         4430          4440          4450          4460          4470
ACT TTC ACC AGC GTT TCT GGG TGA GCA AAA ACA GGA AGG CAA AAT GCC
TGA AAG TGG TCG CAA AGA CCC ACT CGT TTT TGT CCT TCC GTT TTA CGG

             4480          4490          4500          4510
GCA AAA AAG GGA ATA AGG GCG ACA CGG AAA TGT TGA ATA CTC ATA CTC
CGT TTT TTC CCT TAT TCC CGC TGT GCC TTT ACA ACT TAT GAG TAT GAG

4520          4530          4540          4550          4560
TTC CTT TTT CAA TAT TAT TGA AGC ATT TAT CAG GGT TAT TGT CTC ATG
AAG GAA AAA GTT ATA ATA ACT TCG TAA ATA GTC CCA ATA ACA GAG TAC
```

FIG. 4 J

```
  4570      4580      4590       4600      4610
AGC GGA TAC ATA TTT GAA TGT ATT TAG AAA AAT AAA CAA ATA GGG GTT
TCG CCT ATG TAT AAA CTT ACA TAA ATC TTT TTA TTT GTT TAT CCC CAA

  4620      4630      4640      4650       4660
CCG CGC ACA TTT CCC CGA AAA GTG CCA CCT GAC GTC TAA GAA ACC ATT
GGC GCG TGT AAA GGG GCT TTT CAC GGT GGA CTG CAG ATT CTT TGG TAA

    4670      4680       4690      4700      4710
ATT ATC ATG ACA TTA ACC TAT AAA AAT AGG CGT ATC ACG AGG CCC TGA
TAA TAG TAC TGT AAT TGG ATA TTT TTA TCC GCA TAG TGC TCC GGG ACT

      4720      4730      4740       4750
TGG CTC TTT GCG GCA CCC ATC GTT CGT AAT GTT CCG TGG CAC CGA GGA
ACC GAG AAA CGC CGT GGG TAG CAA GCA TTA CAA GGC ACC GTG GCT CCT

4760      4770       4780      4790      4800
CAA CCC TCA AGA GAA AAT GTA ATC ACA CTG GCT CAC CTT CGG GTG GGC
GTT GGG AGT TCT CTT TTA CAT TAG TGT GAC CGA GTG GAA GCC CAC CCG

  4810      4820      4830       4840      4850
CTT TCT GCG TTT ATA AGG AGA CAC TTT ATG TTT AAG AAG GTT GGT AAA
GAA AGA CGC AAA TAT TCC TCT GTG AAA TAC AAA TTC TTC CAA CCA TTT

  4860      4870      4880      4890       4900
TTC CTT GCG GCT TTG GCA GCC AAG CTA GAG ATC TCT AGC TTC GTG TCA
AAG GAA CGC CGA AAC CGT CGG TTC GAT CTC TAG AGA TCG AAG CAC AGT

    4910      4920       4930      4940      4950
AGG ACG GTG ACT GCA GTG AAT AAT AAA ATG TGT GTT TGT CCG AAA TAC
TCC TGC CAC TGA CGT CAC TTA TTA TTT TAC ACA CAA ACA GGC TTT ATG

      4960      4970      4980       4990
GCG TTT TGA GAT TTC TGT CGC CGA CTA AAT TCA TGT CGC GCG ATA GTG
CGC AAA ACT CTA AAG ACA GCG GCT GAT TTA AGT ACA GCG CGC TAT CAC

5000      5010       5020      5030      5040
GTG TTT ATC GCC GAT AGA GAT GGC GAT ATT GGA AAA ATC GAT ATT TGA
CAC AAA TAG CGG CTA TCT CTA CCG CTA TAA CCT TTT TAG CTA TAA ACT

  5050      5060      5070       5080      5090
AAA TAT GGC ATA TTG AAA ATG TCG CCG ATG TGA GTT TCT GTG TAA CTG
TTT ATA CCG TAT AAC TTT TAC AGC GGC TAC ACT CAA AGA CAC ATT GAC
```

FIG. 4 K

```
 5100      5110      5120      5130      5140
ATA TCG CCA TTT TTC CAA AAG TGA TTT TTG GGC ATA CGC GAT ATC TGG
TAT AGC GGT AAA AAG GTT TTC ACT AAA AAC CCG TAT GCG CTA TAG ACC

 5150      5160      5170      5180      5190
CGA TAG CGC TTA TAT CGT TTA CGG GGG ATG GCG ATA GAC GAC TTT GGT
GCT ATC GCG AAT ATA GCA AAT GCC CCC TAC CGC TAT CTG CTG AAA CCA

 5200      5210      5220      5230
GAC TTG GGC GAT TCT GTG TGT CGC AAA TAT CGC AGT TTC GAT ATA GGT
CTG AAC CCG CTA AGA CAC ACA GCG TTT ATA GCG TCA AAG CTA TAT CCA

5240      5250      5260      5270      5280
GAC AGA CGA TAT GAG GCT ATA TCG CCG ATA GAG GCG ACA TCA AGC TGG
CTG TCT GCT ATA CTC CGA TAT AGC GGC TAT CTC CGC TGT AGT TCG ACC

 5290      5300      5310      5320      5330
CAC ATG GCC AAT GCA TAT CGA TCT ATA CAT TGA ATC AAT ATT GGC CAT
GTG TAC CGG TTA CGT ATA GCT AGA TAT GTA ACT TAG TTA TAA CCG GTA

 5340      5350      5360      5370      5380
TAG CCA TAT TAT TCA TTG GTT ATA TAG CAT AAA TCA ATA TTG GCT ATT
ATC GGT ATA ATA AGT AAC CAA TAT ATC GTA TTT AGT TAT AAC CGA TAA

 5390      5400      5410      5420      5430
GGC CAT TGC ATA CGT TGT ATC CAT ATC ATA ATA TGT ACA TTT ATA TTG
CCG GTA ACG TAT GCA ACA TAG GTA TAG TAT TAT ACA TGT AAA TAT AAC

 5440      5450      5460      5470
GCT CAT GTC CAA CAT TAC CGC CAT GTT GAC ATT GAT TAT TGA CTA GTT
CGA GTA CAG GTT GTA ATG GCG GTA CAA CTG TAA CTA ATA ACT GAT CAA

5480      5490      5500      5510      5520
ATT AAT AGT AAT CAA TTA CGG GGT CAT TAG TTC ATA GCC CAT ATA TGG
TAA TTA TCA TTA GTT AAT GCC CCA GTA ATC AAG TAT CGG GTA TAT ACC

 5530      5540      5550      5560      5570
AGT TCC GCG TTA CAT AAC TTA CGG TAA ATG GCC CGC CTG GCT GAC CGC
TCA AGG CGC AAT GTA TTG AAT GCC ATT TAC CGG GCG GAC CGA CTG GCG

 5580      5590      5600      5610      5620
CCA ACG ACC CCC GCC CAT TGA CGT CAA TAA TGA CGT ATG TTC CCA TAG
GGT TGC TGG GGG CGG GTA ACT GCA GTT ATT ACT GCA TAC AAG GGT ATC
```

FIG. 4 L

5630 5640 5650 5660 5670

TAA CGC CAA TAG GGA CTT TCC ATT GAC GTC AAT GGG TGG AGT ATT TAC

ATT GCG GTT ATC CCT GAA AGG TAA CTG CAG TTA CCC ACC TCA TAA ATG

5680 5690 5700 5710

GGT AAA CTG CCC ACT TGG CAG TAC ATC AAG TGT ATC ATA TGC CAA GTA

CCA TTT GAC GGG TGA ACC GTC ATG TAG TTC ACA TAG TAT ACG GTT CAT

5720 5730 5740 5750 5760

CGC CCC CTA TTG ACG TCA ATG ACG GTA AAT GGC CCG CCT GGC ATT ATG

GCG GGG GAT AAC TGC AGT TAC TGC CAT TTA CCG GGC GGA CCG TAA TAC

5770 5780 5790 5800 5810

CCC AGT ACA TGA CCT TAT GGG ACT TTC CTA CTT GGC AGT ACA TCT ACG

GGG TCA TGT ACT GGA ATA CCC TGA AAG GAT GAA CCG TCA TGT AGA TGC

5820 5830 5840 5850 5860

TAT TAG TCA TCG CTA TTA CCA TGG TGA TGC GGT TTT GGC AGT ACA TCA

ATA ATC AGT AGC GAT AAT GGT ACC ACT ACG CCA AAA CCG TCA TGT AGT

5870 5880 5890 5900 5910

ATG GGC GTG GAT AGC GGT TTG ACT CAC GGG GAT TTC CAA GTC TCC ACC

TAC CCG CAC CTA TCG CCA AAC TGA GTG CCC CTA AAG GTT CAG AGG TGG

5920 5930 5940 5950

CCA TTG ACG TCA ATG GGA GTT TGT TTT GGC ACC AAA ATC AAC GGG ACT

GGT AAC TGC AGT TAC CCT CAA ACA AAA CCG TGG TTT TAG TTG CCC TGA

5960 5970 5980 5990 6000

TTC CAA AAT GTC GTA ACA ACT CCG CCC CAT TGA CGC AAA TGG GCG GTA

AAG GTT TTA CAG CAT TGT TGA GGC GGG GTA ACT GCG TTT ACC CGC CAT

6010 6020 6030 6040 6050

GGC GTG TAC GGT GGG AGG TCT ATA TAA GCA GAG CTC GTT TAG TGA ACC

CCG CAC ATG CCA CCC TCC AGA TAT ATT CGT CTC GAG CAA ATC ACT TGG

6060 6070 6080 6090 6100

GTC AGA TCG CCT GGA GAC GCC ATC CAC GCT GTT TTG ACC TCC ATA GAA

CAG TCT AGC GGA CCT CTG CGG TAG GTG CGA CAA AAC TGG AGG TAT CTT

6110 6120 6130 6140 6150

GAC ACC GGG ACC GAT CCA GCC TCC GCG GCC GGG AAC GGT GCA TTG GAA

CTG TGG CCC TGG CTA GGT CGG AGG CGC CGG CCC TTG CCA CGT AAC CTT

FIG. 4 M

```
        6160        6170        6180        6190
          *           *           *           *
CGC GGA TTC CCC GTG CCA AGA GTG ACG TAA GTA CCG CCT ATA GAG TCT
GCG CCT AAG GGG CAC GGT TCT CAC TGC ATT CAT GGC GGA TAT CTC AGA

6200        6210        6220        6230        6240
  *           *           *           *           *
ATA GGC CCA CCC CCT TGG CTT CTT ATG CAT GCT ATA CTG TTT TTG GCT
TAT CCG GGT GGG GGA ACC GAA GAA TAC GTA CGA TAT GAC AAA AAC CGA

   6250        6260        6270        6280        6290
     *           *           *           *           *
TGG GGT CTA TAC ACC CCC GCT TCC TCA TGT TAT AGG TGA TGG TAT AGC
ACC CCA GAT ATG TGG GGG CGA AGG AGT ACA ATA TCC ACT ACC ATA TCG

    6300        6310        6320        6330        6340
      *           *           *           *           *
TTA GCC TAT AGG TGT GGG TTA TTG ACC ATT ATT GAC CAC TCC CCT ATT
AAT CGG ATA TCC ACA CCC AAT AAC TGG TAA TAA CTG GTG AGG GGA TAA

       6350        6360        6370        6380        6390
         *           *           *           *           *
GGT GAC GAT ACT TTC CAT TAC TAA TCC ATA ACA TGG CTC TTT GCC ACA
CCA CTG CTA TGA AAG GTA ATG ATT AGG TAT TGT ACC GAG AAA CGG TGT

          6400        6410        6420        6430
            *           *           *           *
ACT CTC TTT ATT GGC TAT ATG CCA ATA CAC TGT CCT TCA GAG ACT GAC
TGA GAG AAA TAA CCG ATA TAC GGT TAT GTG ACA GGA AGT CTC TGA CTG

6440        6450        6460        6470        6480
  *           *           *           *           *
ACG GAC TCT GTA TTT TTA CAG GAT GGG GTC TCA TTT ATT ATT TAC AAA
TGC CTG AGA CAT AAA AAT GTC CTA CCC CAG AGT AAA TAA TAA ATG TTT

   6490        6500        6510        6520        6530
     *           *           *           *           *
TTC ACA TAT ACA ACA CCA CCG TCC CCA GTG CCC GCA GTT TTT ATT AAA
AAG TGT ATA TGT TGT GGT GGC AGG GGT CAC GGG CGT CAA AAA TAA TTT

    6540        6550        6560        6570        6580
      *           *           *           *           *
CAT AAC GTG GGA TCT CCA CGC GAA TCT CGG GTA CGT GTT CCG GAC ATG
GTA TTG CAC CCT AGA GGT GCG CTT AGA GCC CAT GCA CAA GGC CTG TAC

       6590        6600        6610        6620        6630
         *           *           *           *           *
GGC TCT TCT CCG GTA GCG GCG GAG CTT CTA CAT CCG AGC CCT GCT CCC
CCG AGA AGA GGC CAT CGC CGC CTC GAA GAT GTA GGC TCG GGA CGA GGG

          6640        6650        6660        6670
            *           *           *           *
ATG CCT CCA GCG ACT CAT GGT CGC TCG GCA GCT CCT TGC TCC TAA CAG
TAC GGA GGT CGC TGA GTA CCA GCG AGC CGT CGA GGA ACG AGG ATT GTC
```

FIG. 4 N

6680 6690 6700 6710 6720
TGG AGG CCA GAC TTA GGC ACA GCA CGA TGC CCA CCA CCA CCA GTG TGC
ACC TCC GGT CTG AAT CCG TGT CGT GCT ACG GGT GGT GGT GGT CAC ACG

6730 6740 6750 6760 6770
CGC ACA AGG CCG TGG CGG TAG GGT ATG TGT CTG AAA ATG AGC TCG GGG
GCG TGT TCC GGC ACC GCC ATC CCA TAC ACA GAC TTT TAC TCG AGC CCC

6780 6790 6800 6810 6820
AGC GGG CTT GCA CCG CTG ACG CAT TTG GAA GAC TTA AGG CAG CGG CAG
TCG CCC GAA CGT GGC GAC TGC GTA AAC CTT CTG AAT TCC GTC GCC GTC

6830 6840 6850 6860 6870
AAG AAG ATG CAG GCA GCT GAG TTG TTG TGT TCT GAT AAG AGT CAG AGG
TTC TTC TAC GTC CGT CGA CTC AAC AAC ACA AGA CTA TTC TCA GTC TCC

6880 6890 6900 6910
TAA CTC CCG TTG CGG TGC TGT TAA CGG TGG AGG GCA GTG TAG TCT GAG
ATT GAG GGC AAC GCC ACG ACA ATT GCC ACC TCC CGT CAC ATC AGA CTC

6920 6930 6940 6950 6960
CAG TAC TCG TTG CTG CCG CGC GCG CCA CCA GAC ATA ATA GCT GAC AGA
GTC ATG AGC AAC GAC GGC GCG CGC GGT GGT CTG TAT TAT CGA CTG TCT

6970 6980 6990 7000 7010
CTA ACA GAC TGT TCC TTT CCA TGG GTC TTT TCT GCA GTC ACC GTC CTT
GAT TGT CTG ACA AGG AAA GGT ACC CAG AAA AGA CGT CAG TGG CAG GAA

7020 7030 7040 7050 7060
GAC ACG AAG CTT GGG CTG CAG GTC GAT CGA CTC TAG AGG ATC GAT CCC
CTG TGC TTC GAA CCC GAC GTC CAG CTA GCT GAG ATC TCC TAG CTA GGG

7070
CGG GCG AGC TC
GCC CGC TCG AG

FIG. 5 A

The pEe12TF8LCDR3 expression vector DNA sequence. The coding regions of the TF8-5G9 CDR-grafted LC gene, TF8LCDR3, are translated.

Sequence Range: 1 to 7864

```
                   10          20          30          40          50
                   *           *           *           *           *
AAT TCA CC ATG GGT GTG CCA ACT CAG GTA TTA GGA TTA CTG CTG CTG TGG
TTA AGT GG TAC CCA CAC GGT TGA GTC CAT AAT CCT AAT GAC GAC GAC ACC
           Met Gly Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp>

                   60          70          80          90
                   *           *           *           *
CTT ACA GAT GCA AGA TGT GAT ATC CAA ATG ACA CAA TCT CCT TCT TCT
GAA TGT CTA CGT TCT ACA CTA TAG GTT TAC TGT GTT AGA GGA AGA AGA
Leu Thr Asp Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser>

100         110         120         130         140
*           *           *           *           *
CTA AGT GCT TCT GTC GGA GAT AGA GTA ACA ATT ACA TGT AAG GCG AGT
GAT TCA CGA AGA CAG CCT CTA TCT CAT TGT TAA TGT ACA TTC CGC TCA
Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser>

   150         160         170         180         190
   *           *           *           *           *
CAG GAC ATT AGA AAG TAT TTA AAC TGG TAT CAG CAA AAA CCT GGG AAG
GTC CTG TAA TCT TTC ATA AAT TTG ACC ATA GTC GTT TTT GGA CCC TTC
Gln Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys>

      200         210         220         230         240
      *           *           *           *           *
GCT CCT AAG CTA CTG ATT TAT TAT GCA ACA AGT TTG GCA GAT GGA GTA
CGA GGA TTC GAT GAC TAA ATA ATA CGT TGT TCA AAC CGT CTA CCT CAT
Ala Pro Lys Leu Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val>

         250         260         270         280         290
         *           *           *           *           *
CCT TCT AGA TTT TCT GGT TCT GGC TCT GGA ACA GAC TAC ACA TTC ACA
GGA AGA TCT AAA AGA CCA AGA CCG AGA CCT TGT CTG ATG TGT AAG TGT
Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr>

               300         310         320         330
               *           *           *           *
ATT TCT TCT CTC CAA CCT GAG GAC ATT GCT ACA TAC TAC TGC CTA CAA
TAA AGA AGA GAG GTT GGA CTC CTG TAA CGA TGT ATG ATG ACG GAT GTT
Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln>

340         350         360         370         380
*           *           *           *           *
CAT GGT GAG AGT CCG TAT ACA TTT GGA CAA GGA ACA AAA CTA GAG ATC
GTA CCA CTC TCA GGC ATA TGT AAA CCT GTT CCT TGT TTT GAT CTC TAG
His Gly Glu Ser Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile>

   390         400         410         420         430
   *           *           *           *           *
ACA AGA ACT GTT GCG GCG CCG TCT GTC TTC ATC TTC CCG CCA TCT GAT
TGT TCT TGA CAA CGC CGC GGC AGA CAG AAG TAG AAG GGC GGT AGA CTA
Thr Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp>
```

FIG. 5 B

```
      440             450             460             470             480
GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC
CTC GTC AAC TTT AGA CCT TGA CGG AGA CAA CAC ACG GAC GAC TTA TTG
Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn>

          490             500             510             520             530
TTC TAT CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC
AAG ATA GGG TCT CTC CGG TTT CAT GTC ACC TTC CAC CTA TTG CGG GAG
Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu>

              540             550             560             570
CAA TCG GGT AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC
GTT AGC CCA TTG AGG GTC CTC TCA CAG TGT CTC GTC CTG TCG TTC CTG
Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp>

580             590             600             610             620
AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC
TCG TGG ATG TCG GAG TCG TCG TGG GAC TGC GAC TCG TTT CGT CTG ATG
Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr>

   630             640             650             660             670
GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC
CTC TTT GTG TTT CAG ATG CGG ACG CTT CAG TGG GTA GTC CCG GAC TCG
Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser>

       680             690             700             710             720
TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT T AGA GGG AGA AGT
AGC GGG CAG TGT TTC TCG AAG TTG TCC CCT CTC ACA A TCT CCC TCT TCA
Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys>

          730             740             750             760             770
GCC CCC ACC TGC TCC TCA GTT CCA GCC TGG GGA TCA TAA TCA GCC ATA
CGG GGG TGG ACG AGG AGT CAA GGT CGG ACC CCT AGT ATT AGT CGG TAT

              780             790             800             810
CCA CAT TTG TAG AGG TTT TAC TTG CTT TAA AAA ACC TCC CAC ACC TCC
GGT GTA AAC ATC TCC AAA ATG AAC GAA ATT TTT TGG AGG GTG TGG AGG

820             830             840             850             860
CCC TGA ACC TGA AAC ATA AAA TGA ATG CAA TTG TTG TTG TTA ACT TGT
GGG ACT TGG ACT TTG TAT TTT ACT TAC GTT AAC AAC AAC AAT TGA ACA

870             880             890             900             910
TTA TTG CAG CTT ATA ATG GTT ACA AAT AAA GCA ATA GCA TCA CAA ATT
AAT AAC GTC GAA TAT TAC CAA TGT TTA TTT CGT TAT CGT AGT GTT TAA
```

FIG. 5 C

```
     920       930       940       950       960
TCA CAA ATA AAG CAT TTT TTT CAC TGC ATT CTA GTT GTG GTT TGT CCA
AGT GTT TAT TTC GTA AAA AAA GTG ACG TAA GAT CAA CAC CAA ACA GGT

     970       980       990      1000      1010
AAC TCA TCA ATG TAT CTT ATC ATG TCT GGA TCC TCT ACG CCG GAC GCA
TTG AGT AGT TAC ATA GAA TAG TAC AGA CCT AGG AGA TGC GGC CTG CGT

    1020      1030      1040      1050
TCG TGG CCG GCA TCA CCG GCG CCA CAG GTG CGG TTG CTG GCG CCT ATA
AGC ACC GGC CGT AGT GGC CGC GGT GTC CAC GCC AAC GAC CGC GGA TAT

1060      1070      1080      1090      1100
TCG CCG ACA TCA CCG ATG GGG AAG ATC GGG CTC GCC ACT TCG GGC TCA
AGC GGC TGT AGT GGC TAC CCC TTC TAG CCC GAG CGG TGA AGC CCG AGT

1110      1120      1130      1140      1150
TGA GCG CTT GTT TCG GCG TGG GTA TGG TGG CAG GCC CGT GGC CGG GGG
ACT CGC GAA CAA AGC CGC ACC CAT ACC ACC GTC CGG GCA CCG GCC CCC

    1160      1170      1180      1190      1200
ACT GTT GGG CGC CAT CTC CTT GCA TGC ACC ATT CCT TGC GGC GGC GGT
TGA CAA CCC GCG GTA GAG GAA CGT ACG TGG TAA GGA ACG CCG CCG CCA

    1210      1220      1230      1240      1250
GCT CAA CGG CCT CAA CCT ACT ACT GGG CTG CTT CCT AAT GCA GGA GTC
CGA GTT GCC GGA GTT GGA TGA TGA CCC GAC GAA GGA TTA CGT CCT CAG

    1260      1270      1280      1290
GCA TAA GGG AGA GCG TCG ACC TCG GGC CGC GTT GCT GGC GTT TTT CCA
CGT ATT CCC TCT CGC AGC TGG AGC CCG GCG CAA CGA CCG CAA AAA GGT

1300      1310      1320      1330      1340
TAG GCT CCG CCC CCC TGA CGA GCA TCA CAA AAA TCG ACG CTC AAG TCA
ATC CGA GGC GGG GGG ACT GCT CGT AGT GTT TTT AGC TGC GAG TTC AGT

1350      1360      1370      1380      1390
GAG GTG GCG AAA CCC GAC AGG ACT ATA AAG ATA CCA GGC GTT TCC CCC
CTC CAC CGC TTT GGG CTG TCC TGA TAT TTC TAT GGT CCG CAA AGG GGG

    1400      1410      1420      1430      1440
TGG AAG CTC CCT CGT GCG CTC TCC TGT TCC GAC CCT GCC GCT TAC CGG
ACC TTC GAG GGA GCA CGC GAG AGG ACA AGG CTG GGA CGG CGA ATG GCC

    1450      1460      1470      1480      1490
ATA CCT GTC CGC CTT TCT CCC TTC GGG AAG CGT GGC GCT TTC TCA ATG
TAT GGA CAG GCG GAA AGA GGG AAG CCC TTC GCA CCG CGA AAG AGT TAC
```

FIG. 5 D

```
        1500        1510        1520        1530
CTC ACG CTG TAG GTA TCT CAG TTC GGT GTA GGT CGT TCG CTC CAA GCT
GAG TGC GAC ATC CAT AGA GTC AAG CCA CAT CCA GCA AGC GAG GTT CGA

1540        1550        1560        1570        1580
GGG CTG TGT GCA CGA ACC CCC CGT TCA GCC CGA CCG CTG CGC CTT ATC
CCC GAC ACA CGT GCT TGG GGG GCA AGT CGG GCT GGC GAC GCG GAA TAG

 1590        1600        1610        1620        1630
CGG TAA CTA TCG TCT TGA GTC CAA CCC GGT AAG ACA CGA CTT ATC GCC
GCC ATT GAT AGC AGA ACT CAG GTT GGG CCA TTC TGT GCT GAA TAG CGG

    1640        1650        1660        1670        1680
ACT GGC AGC AGC CAC TGG TAA CAG GAT TAG CAG AGC GAG GTA TGT AGG
TGA CCG TCG TCG GTG ACC ATT GTC CTA ATC GTC TCG CTC CAT ACA TCC

      1690        1700        1710        1720        1730
CGG TGC TAC AGA GTT CTT GAA GTG GTG GCC TAA CTA CGG CTA CAC TAG
GCC ACG ATG TCT CAA GAA CTT CAC CAC CGG ATT GAT GCC GAT GTG ATC

        1740        1750        1760        1770
AAG GAC AGT ATT TGG TAT CTG CGC TCT GCT GAA GCC AGT TAC CTT CGG
TTC CTG TCA TAA ACC ATA GAC GCG AGA CGA CTT CGG TCA ATG GAA GCC

1780        1790        1800        1810        1820
AAA AAG AGT TGG TAG CTC TTG ATC CGG CAA ACA AAC CAC CGC TGG TAG
TTT TTC TCA ACC ATC GAG AAC TAG GCC GTT TGT TTG GTG GCG ACC ATC

 1830        1840        1850        1860        1870
CGG TGG TTT TTT TGT TTG CAA GCA GCA GAT TAC GCG CAG AAA AAA AGG
GCC ACC AAA AAA ACA AAC GTT CGT CGT CTA ATG CGC GTC TTT TTT TCC

    1880        1890        1900        1910        1920
ATC TCA AGA AGA TCC TTT GAT CTT TTC TAC GGG GTC TGA CGC TCA GTG
TAG AGT TCT TCT AGG AAA CTA GAA AAG ATG CCC CAG ACT GCG AGT CAC

      1930        1940        1950        1960        1970
GAA CGA AAA CTC ACG TTA AGG GAT TTT GGT CAT GAG ATT ATC AAA AAG
CTT GCT TTT GAG TGC AAT TCC CTA AAA CCA GTA CTC TAA TAG TTT TTC

        1980        1990        2000        2010
GAT CTT CAC CTA GAT CCT TTT AAA TTA AAA ATG AAG TTT TAA ATC AAT
CTA GAA GTG GAT CTA GGA AAA TTT AAT TTT TAC TTC AAA ATT TAG TTA

2020        2030        2040        2050        2060
CTA AAG TAT ATA TGA GTA AAC TTG GTC TGA CAG TTA CCA ATG CTT AAT
GAT TTC ATA TAT ACT CAT TTG AAC CAG ACT GTC AAT GGT TAC GAA TTA
```

FIG. 5 E

```
2070        2080        2090        2100        2110
CAG TGA GGC ACC TAT CTC AGC GAT CTG TCT ATT TCG TTC ATC CAT AGT
GTC ACT CCG TGG ATA GAG TCG CTA GAC AGA TAA AGC AAG TAG GTA TCA

2120        2130        2140        2150        2160
TGC CTG ACT CCC CGT CGT GTA GAT AAC TAC GAT ACG GGA GGG CTT ACC
ACG GAC TGA GGG GCA GCA CAT CTA TTG ATG CTA TGC CCT CCC GAA TGG

2170        2180        2190        2200        2210
ATC TGG CCC CAG TGC TGC AAT GAT ACC GCG AGA CCC ACG CTC ACC GGC
TAG ACC GGG GTC ACG ACG TTA CTA TGG CGC TCT GGG TGC GAG TGG CCG

2220        2230        2240        2250
TCC AGA TTT ATC AGC AAT AAA CCA GCC AGC CGG AAG GGC CGA GCG CAG
AGG TCT AAA TAG TCG TTA TTT GGT CGG TCG GCC TTC CCG GCT CGC GTC

2260        2270        2280        2290        2300
AAG TGG TCC TGC AAC TTT ATC CGC CTC CAT CCA GTC TAT TAA TTG TTG
TTC ACC AGG ACG TTG AAA TAG GCG GAG GTA GGT CAG ATA ATT AAC AAC

2310        2320        2330        2340        2350
CCG GGA AGC TAG AGT AAG TAG TTC GCC AGT TAA TAG TTT GCG CAA CGT
GGC CCT TCG ATC TCA TTC ATC AAG CGG TCA ATT ATC AAA CGC GTT GCA

2360        2370        2380        2390        2400
TGT TGC CAT TGC TAC AGG CAT CGT GGT GTC ACG CTC GTC GTT TGG TAT
ACA ACG GTA ACG ATG TCC GTA GCA CCA CAG TGC GAG CAG CAA ACC ATA

2410        2420        2430        2440        2450
GGC TTC ATT CAG CTC CGG TTC CCA ACG ATC AAG GCG AGT TAC ATG ATC
CCG AAG TAA GTC GAG GCC AAG GGT TGC TAG TTC CGC TCA ATG TAC TAG

2460        2470        2480        2490
CCC CAT GTT GTG CAA AAA AGC GGT TAG CTC CTT CGG TCC TCC GAT CGT
GGG GTA CAA CAC GTT TTT TCG CCA ATC GAG GAA GCC AGG AGG CTA GCA

2500        2510        2520        2530        2540
TGT CAG AAG TAA GTT GGC CGC AGT GTT ATC ACT CAT GGT TAT GGC AGC
ACA GTC TTC ATT CAA CCG GCG TCA CAA TAG TGA GTA CCA ATA CCG TCG

2550        2560        2570        2580        2590
ACT GCA TAA TTC TCT TAC TGT CAT GCC ATC CGT AAG ATG CTT TTC TGT
TGA CGT ATT AAG AGA ATG ACA GTA CGG TAG GCA TTC TAC GAA AAG ACA

2600        2610        2620        2630        2640
GAC TGG TGA GTA CTC AAC CAA GTC ATT CTG AGA ATA GTG TAT GCG GCG
CTG ACC ACT CAT GAG TTG GTT CAG TAA GAC TCT TAT CAC ATA CGC CGC
```

FIG. 5 F

```
      2650        2660        2670        2680        2690
ACC GAG TTG CTC TTG CCC GGC GTC AAC ACG GGA TAA TAC CGC GCC ACA
TGG CTC AAC GAG AAC GGG CCG CAG TTG TGC CCT ATT ATG GCG CGG TGT

      2700        2710        2720        2730
TAG CAG AAC TTT AAA AGT GCT CAT CAT TGG AAA ACG TTC TTC GGG GCG
ATC GTC TTG AAA TTT TCA CGA GTA GTA ACC TTT TGC AAG AAG CCC CGC

2740        2750        2760        2770        2780
AAA ACT CTC AAG GAT CTT ACC GCT GTT GAG ATC CAG TTC GAT GTA ACC
TTT TGA GAG TTC CTA GAA TGG CGA CAA CTC TAG GTC AAG CTA CAT TGG

  2790        2800        2810        2820        2830
CAC TCG TGC ACC CAA CTG ATC TTC AGC ATC TTT TAC TTT CAC CAG CGT
GTG AGC ACG TGG GTT GAC TAG AAG TCG TAG AAA ATG AAA GTG GTC GCA

    2840        2850        2860        2870        2880
TTC TGG GTG AGC AAA AAC AGG AAG GCA AAA TGC CGC AAA AAA GGG AAT
AAG ACC CAC TCG TTT TTG TCC TTC CGT TTT ACG GCG TTT TTT CCC TTA

      2890        2900        2910        2920        2930
AAG GGC GAC ACG GAA ATG TTG AAT ACT CAT ACT CTT CCT TTT TCA ATA
TTC CCG CTG TGC CTT TAC AAC TTA TGA GTA TGA GAA GGA AAA AGT TAT

      2940        2950        2960        2970
TTA TTG AAG CAT TTA TCA GGG TTA TTG TCT CAT GAG CGG ATA CAT ATT
AAT AAC TTC GTA AAT AGT CCC AAT AAC AGA GTA CTC GCC TAT GTA TAA

2980        2990        3000        3010        3020
TGA ATG TAT TTA GAA AAA TAA ACA AAT AGG GGT TCC GCG CAC ATT TCC
ACT TAC ATA AAT CTT TTT ATT TGT TTA TCC CCA AGG CGC GTG TAA AGG

3030        3040        3050        3060        3070
CCG AAA AGT GCC ACC TGA CGT CTA AGA AAC CAT TAT TAT CAT GAC ATT
GGC TTT TCA CGG TGG ACT GCA GAT TCT TTG GTA ATA ATA GTA CTG TAA

    3080        3090        3100        3110        3120
AAC CTA TAA AAA TAG GCG TAT CAC GAG GCC CTG ATG GCT CTT TGC GCC
TTG GAT ATT TTT ATC CGC ATA GTG CTC CGG GAC TAC CGA GAA ACG CGG

      3130        3140        3150        3160        3170
ACC CAT CGT TCG TAA TGT TCC GTG GCA CCG AGG ACA ACC CTC AAG AGA
TGG GTA GCA AGC ATT ACA AGG CAC CGT GGC TCC TGT TGG GAG TTC TCT

        3180        3190        3200        3210
AAA TGT AAT CAC ACT GGC TCA CCT TCG GGT GGG CCT TTC TGC GTT TAT
TTT ACA TTA GTG TGA CCG AGT GGA AGC CCA CCC GGA AAG ACG CAA ATA
```

FIG. 5 G

3220 3230 3240 3250 3260
AAG GAG ACA CTT TAT GTT TAA GAA GGT TGG TAA ATT CCT TGC GGC TTT
TTC CTC TGT GAA ATA CAA ATT CTT CCA ACC ATT TAA GGA ACG CCG AAA

3270 3280 3290 3300 3310
GGC AGC CAA GCT AGA GAT CCG GCT GTG GAA TGT GTG TCA GTT AGG GTG
CCG TCG GTT CGA TCT CTA GGC CGA CAC CTT ACA CAC AGT CAA TCC CAC

3320 3330 3340 3350 3360
TGG AAA GTC CCC AGG CTC CCC AGC AGG CAG AAG TAT GCA AAG CAT GCA
ACC TTT CAG GGG TCC GAG GGG TCG TCC GTC TTC ATA CGT TTC GTA CGT

3370 3380 3390 3400 3410
TCT CAA TTA GTC AGC AAC CAG GCT CCC CAG CAG GCA GAA GTA TGC AAA
AGA GTT AAT CAG TCG TTG GTC CGA GGG GTC GTC CGT CTT CAT ACG TTT

3420 3430 3440 3450
GCA TGC ATC TCA ATT AGT CAG CAA CCA TAG TCC CGC CCC TAA CTC CGC
CGT ACG TAG AGT TAA TCA GTC GTT GGT ATC AGG GCG GGG ATT GAG GCG

3460 3470 3480 3490 3500
CCA TCC CGC CCC TAA CTC CGC CCA GTT CCG CCC ATT CTC CGC CCC ATG
GGT AGG GCG GGG ATT GAG GCG GGT CAA GGC GGG TAA GAG GCG GGG TAC

3510 3520 3530 3540 3550
GCT GAC TAA TTT TTT TTA TTT ATG CAG AGG CCG AGG CCG CCT CGG CCT
CGA CTG ATT AAA AAA AAT AAA TAC GTC TCC GGC TCC GGC GGA GCC GGA

3560 3570 3580 3590 3600
CTG AGC TAT TCC AGA AGT AGT GAG GAG GCT TTT TTG GAG GCC TAG GCT
GAC TCG ATA AGG TCT TCA TCA CTC CTC CGA AAA AAC CTC CGG ATC CGA

3610 3620 3630 3640 3650
TTT GCA AAA AGC TAG CTT GGG GCC ACC GCT CAG AGC ACC TTC CAC CAT
AAA CGT TTT TCG ATC GAA CCC CGG TGG CGA GTC TCG TGG AAG GTG GTA

3660 3670 3680 3690
GGC CAC CTC AGC AAG TTC CCA CTT GAA CAA AAA CAT CAA GCA AAT GTA
CCG GTG GAG TCG TTC AAG GGT GAA CTT GTT TTT GTA GTT CGT TTA CAT

3700 3710 3720 3730 3740
CTT GTG CCT GCC CCA GGG TGA GAA AGT CCA AGC CAT GTA TAT CTG GGT
GAA CAC GGA CGG GGT CCC ACT CTT TCA GGT TCG GTA CAT ATA GAC CCA

3750 3760 3770 3780 3790
TGA TGG TAC TGG AGA AGG ACT GCG CTG CAA AAC CCG CAC CCT GGA CTG
ACT ACC ATG ACC TCT TCC TGA CGC GAC GTT TTG GGC GTG GGA CCT GAC

FIG. 5 H

3800 3810 3820 3830 3840
TGA GCC CAA GTG TGT AGA AGA GTT ACC TGA GTG GAA TTT TGA TGG CTC
ACT CGG GTT CAC ACA TCT TCT CAA TGG ACT CAC CTT AAA ACT ACC GAG

3850 3860 3870 3880 3890
TAG TAC CTT TCA GTC TGA GGG CTC CAA CAG TGA CAT GTA TCT CAG CCC
ATC ATG GAA AGT CAG ACT CCC GAG GTT GTC ACT GTA CAT AGA GTC GGG

3900 3910 3920 3930
TGT TGC CAT GTT TCG GGA CCC CTT CCG CAG AGA TCC CAA CAA GCT GGT
ACA ACG GTA CAA AGC CCT GGG GAA GGC GTC TCT AGG GTT GTT CGA CCA

3940 3950 3960 3970 3980
GTT CTG TGA AGT TTT CAA GTA CAA CCG GAA GCC TGC AGA GAC CAA TTT
CAA GAC ACT TCA AAA GTT CAT GTT GGC CTT CGG ACG TCT CTG GTT AAA

3990 4000 4010 4020 4030
AAG GCA CTC GTG TAA ACG GAT AAT GGA CAT GGT GAG CAA CCA GCA CCC
TTC CGT GAG CAC ATT TGC CTA TTA CCT GTA CCA CTC GTT GGT CGT GGG

4040 4050 4060 4070 4080
CTG GTT TGG AAT GGA ACA GGA GTA TAC TCT GAT GGG AAC AGA TGG GCA
GAC CAA ACC TTA CCT TGT CCT CAT ATG AGA CTA CCC TTG TCT ACC CGT

4090 4100 4110 4120 4130
CCC TTT TGG TTG GCC TTC CAA TGG CTT TCC TGG GCC CCA AGG TCC GTA
GGG AAA ACC AAC CGG AAG GTT ACC GAA AGG ACC CGG GGT TCC AGG CAT

4140 4150 4160 4170
TTA CTG TGG TGT GGG CGC AGA CAA AGC CTA TGG CAG GGA TAT CGT GGA
AAT GAC ACC ACA CCC GCG TCT GTT TCG GAT ACC GTC CCT ATA GCA CCT

4180 4190 4200 4210 4220
GGC TCA CTA CCG CGC CTG CTT GTA TGC TGG GGT CAA GAT TAC AGG AAC
CCG AGT GAT GGC GCG GAC GAA CAT ACG ACC CCA GTT CTA ATG TCC TTG

4230 4240 4250 4260 4270
AAA TGC TGA GGT CAT GCC TGC CCA GTG GGA ACT CCA AAT AGG ACC CTG
TTT ACG ACT CCA GTA CGG ACG GGT CAC CCT TGA GGT TTA TCC TGG GAC

4280 4290 4300 4310 4320
TGA AGG AAT CCG CAT GGG AGA TCA TCT CTG GGT GGC CCG TTT CAT CTT
ACT TCC TTA GGC GTA CCC TCT AGT AGA GAC CCA CCG GGC AAA GTA GAA

4330 4340 4350 4360 4370
NCA TCG AGT ATG TGA AGA CTT TGG GGT AAT AGC AAC CTT TGA CCC CAA
NGT AGC TCA TAC ACT TCT GAA ACC CCA TTA TCG TTG GAA ACT GGG GTT

FIG. 5 I

```
        4380            4390          4400           4410
GCC CAT TCC TGG GAA CTG GAA TGG TGC AGG CTG CCA TAC CAA CTT TAG
CGG GTA AGG ACC CTT GAC CTT ACC ACG TCC GAC GGT ATG GTT GAA ATC

4420        4430           4440          4450           4460
CAC CAA GGC CAT GCG GGA GGA GAA TGG TCT GAA GCA CAT CGA GGA GGC
GTG GTT CCG GTA CGC CCT CCT CTT ACC AGA CTT CGT GTA GCT CCT CCG

 4470         4480          4490           4500          4510
CAT CGA GAA ACT AAG CAA GCG GCA CCG GTA CCA CAT TCG AGC CTA CGA
GTA GCT CTT TGA TTC GTT CGC CGT GGC CAT GGT GTA AGC TCG GAT GCT

    4520         4530           4540          4550          4560
TCC CAA GGG GGG CCT GGA CAA TGC CCG TGG TCT GAC TGG GTT CCA CGA
AGG GTT CCC CCC GGA CCT GTT ACG GGC ACC AGA CTG ACC CAA GGT GCT

       4570          4580          4590           4600          4610
AAC GTC CAA CAT CAA CGA CTT TTC TGC TGG TGT CGC CAA TCG CAG TGC
TTG CAG GTT GTA GTT GCT GAA AAG ACG ACC ACA GCG GTT AGC GTC ACG

          4620           4630          4640          4650
CAG CAT CCG CAT TCC CCG GAC TGT CGG CCA GGA GAA GAA AGG TTA CTT
GTC GTA GGC GTA AGG GGC CTG ACA GCC GGT CCT CTT CTT TCC AAT GAA

4660         4670          4680           4690          4700
TGA AGA CCG CGG CCC CTC TGC CAA TTG TGA CCC CTT TGC AGT GAC AGA
ACT TCT GGC GCC GGG GAG ACG GTT AAC ACT GGG GAA ACG TCA CTG TCT

 4710           4720          4730          4740           4750
AGC CAT CGT CCG CAC ATG CCT TCT CAA TGA GAC TGG CCA CGA GCC CTT
TCG GTA GCA GGC GTG TAC GGA AGA GTT ACT CTG ACC GGT GCT CGG GAA

    4760          4770           4780          4790          4800
CCA ATA CAA AAA CTA ATT AGA CTT TGA GTG ATC TTG AGC CTT TCC TAG
GGT TAT GTT TTT GAT TAA TCT GAA ACT CAC TAG AAC TCG GAA AGG ATC

       4810          4820          4830           4840          4850
TTC ATC CCA CCC CGC CCC AGA GAG ATC TTT GTG AAG GAA CCT TAC TTC
AAG TAG GGT GGG GCG GGG TCT CTC TAG AAA CAC TTC CTT GGA ATG AAG

          4860          4870          4880          4890
TGT GGT GTG ACA TAA TTG GAC AAA CTA CCT ACA GAG ATT TAA AGC TCT
ACA CCA CAC TGT ATT AAC CTG TTT GAT GGA TGT CTC TAA ATT TCG AGA

4900         4910          4920           4930          4940
AAG GTA AAT ATA AAA TTT TTA AGT GTA TAA TGT GTT AAA CTA CTG ATT
TTC CAT TTA TAT TTT AAA AAT TCA CAT ATT ACA CAA TTT GAT GAC TAA
```

FIG. 5 J

4950 4960 4970 4980 4990
CTA ATT GTT TGT GTA TTT TAG ATT CCA ACC TAT GGA ACT GAT GAA TGG
GAT TAA CAA ACA CAT AAA ATC TAA GGT TGG ATA CCT TGA CTA CTT ACC

5000 5010 5020 5030 5040
GAG CAG TGG TGG AAT GCC TTT AAT GAG GAA AAC CTG TTT TGC TCA GAA
CTC GTC ACC ACC TTA CGG AAA TTA CTC CTT TTG GAC AAA ACG AGT CTT

5050 5060 5070 5080 5090
GAA ATG CCA TCT AGT GAT GAT GAG GCT ACT GCT GAC TCT CAA CAT TCT
CTT TAC GGT AGA TCA CTA CTA CTC CGA TGA CGA CTG AGA GTT GTA AGA

5100 5110 5120 5130
ACT CCT CCA AAA AAG AAG AGA AAG GTA GAA GAC CCC AAG GAC TTT CCT
TGA GGA GGT TTT TTC TTC TCT TTC CAT CTT CTG GGG TTC CTG AAA GGA

5140 5150 5160 5170 5180
TCA GAA TTG CTA AGT TTT TTG AGT CAT GCT GTG TTT AGT AAT AGA ACT
AGT CTT AAC GAT TCA AAA AAC TCA GTA CGA CAC AAA TCA TTA TCT TGA

5190 5200 5210 5220 5230
CTT GCT TGC TTT GCT ATT TAC ACC ACA AAG GAA AAA GCT GCA CTG CTA
GAA CGA ACG AAA CGA TAA ATG TGG TGT TTC CTT TTT CGA CGT GAC GAT

5240 5250 5260 5270 5280
TAC AAG AAA ATT ATG GAA AAA TAT TCT GTA ACC TTT ATA AGT AGG CAT
ATG TTC TTT TAA TAC CTT TTT ATA AGA CAT TGG AAA TAT TCA TCC GTA

5290 5300 5310 5320 5330
AAC AGT TAT AAT CAT AAC ATA CTG TTT TTT CTT ACT CCA CAC AGG CAT
TTG TCA ATA TTA GTA TTG TAT GAC AAA AAA GAA TGA GGT GTG TCC GTA

5340 5350 5360 5370
AGA GTG TCT GCT ATT AAT AAC TAT GCT CAA AAA TTG TGT ACC TTT AGC
TCT CAC AGA CGA TAA TTA TTG ATA CGA GTT TTT AAC ACA TGG AAA TCG

5380 5390 5400 5410 5420
TTT TTA ATT TGT AAA GGG GTT AAT AAG GAA TAT TTG ATG TAT AGT GCC
AAA AAT TAA ACA TTT CCC CAA TTA TTC CTT ATA AAC TAC ATA TCA CGG

5430 5440 5450 5460 5470
TTG ACT AGA GAT CAT AAT CAG CCA TAC CAC ATT TGT AGA GGT TTT ACT
AAC TGA TCT CTA GTA TTA GTC GGT ATG GTG TAA ACA TCT CCA AAA TGA

5480 5490 5500 5510 5520
TGC TTT AAA AAA CCT CCC ACA CCT CCC CCT GAA CCT GAA ACA TAA AAT
ACG AAA TTT TTT GGA GGG TGT GGA GGG GGA CTT GGA CTT TGT ATT TTA

FIG. 5 K

```
      5530      5540      5550      5560      5570
GAA TGC AAT TGT TGT TGT TAA CTT GTT TAT TGC AGC TTA TAA TGG TTA
CTT ACG TTA ACA ACA ACA ATT GAA CAA ATA ACG TCG AAT ATT ACC AAT

      5580      5590      5600      5610
CAA ATA AAG CAA TAG CAT CAC AAA TTT CAC AAA TAA AGC ATT TTT TTC
GTT TAT TTC GTT ATC GTA GTG TTT AAA GTG TTT ATT TCG TAA AAA AAG

5620      5630      5640      5650      5660
ACT GCA TTC TAG TTG TGG TTT GTC CAA ACT CAT CAA TGT ATC TTA TCA
TGA CGT AAG ATC AAC ACC AAA CAG GTT TGA GTA GTT ACA TAG AAT AGT

5670      5680      5690      5700      5710
TGT CTG GAT CTC TAG CTT CGT GTC AAG GAC GGT GAC TGC AGT GAA TAA
ACA GAC CTA GAG ATC GAA GCA CAG TTC CTG CCA CTG ACG TCA CTT ATT

5720      5730      5740      5750      5760
TAA AAT GTG TGT TTG TCC GAA ATA CGC GTT TTG AGA TTT CTG TCG CCG
ATT TTA CAC ACA AAC AGG CTT TAT GCG CAA AAC TCT AAA GAC AGC GGC

      5770      5780      5790      5800      5810
ACT AAA TTC ATG TCG CGC GAT AGT GGT GTT TAT CGC CGA TAG AGA TGG
TGA TTT AAG TAC AGC GCG CTA TCA CCA CAA ATA GCG GCT ATC TCT ACC

      5820      5830      5840      5850
CGA TAT TGG AAA AAT CGA TAT TTG AAA ATA TGG CAT ATT GAA AAT GTC
GCT ATA ACC TTT TTA GCT ATA AAC TTT TAT ACC GTA TAA CTT TTA CAG

5860      5870      5880      5890      5900
GCC GAT GTG AGT TTC TGT GTA ACT GAT ATC GCC ATT TTT CCA AAA GTG
CGG CTA CAC TCA AAG ACA CAT TGA CTA TAG CGG TAA AAA GGT TTT CAC

5910      5920      5930      5940      5950
ATT TTT GGG CAT ACG CGA TAT CTG GCG ATA GCG CTT ATA TCG TTT ACG
TAA AAA CCC GTA TGC GCT ATA GAC CGC TAT CGC GAA TAT AGC AAA TGC

5960      5970      5980      5990      6000
GGG GAT GGC GAT AGA CGA CTT TGG TGA CTT GGG CGA TTC TGT GTG TCG
CCC CTA CCG CTA TCT GCT GAA ACC ACT GAA CCC GCT AAG ACA CAC AGC

      6010      6020      6030      6040      6050
CAA ATA TCG CAG TTT CGA TAT AGG TGA CAG ACG ATA TGA GGC TAT ATC
GTT TAT AGC GTC AAA GCT ATA TCC ACT GTC TGC TAT ACT CCG ATA TAG

      6060      6070      6080      6090
GCC GAT AGA GGC GAC ATC AAG CTG GCA CAT GGC CAA TGC ATA TCG ATC
CGG CTA TCT CCG CTG TAG TTC GAC CGT GTA CCG GTT ACG TAT AGC TAG
```

FIG. 5 L

```
6100        6110        6120        6130        6140
TAT ACA TTG AAT CAA TAT TGG CCA TTA GCC ATA TTA TTC ATT GGT TAT
ATA TGT AAC TTA GTT ATA ACC GGT AAT CGG TAT AAT AAG TAA CCA ATA

6150        6160        6170        6180        6190
ATA GCA TAA ATC AAT ATT GGC TAT TGG CCA TTG CAT ACG TTG TAT CCA
TAT CGT ATT TAG TTA TAA CCG ATA ACC GGT AAC GTA TGC AAC ATA GGT

6200        6210        6220        6230        6240
TAT CAT AAT ATG TAC ATT TAT ATT GGC TCA TGT CCA ACA TTA CCG CCA
ATA GTA TTA TAC ATG TAA ATA TAA CCG AGT ACA GGT TGT AAT GGC GGT

6250        6260        6270        6280        6290
TGT TGA CAT TGA TTA TTG ACT AGT TAT TAA TAG TAA TCA ATT ACG GGG
ACA ACT GTA ACT AAT AAC TGA TCA ATA ATT ATC ATT AGT TAA TGC CCC

6300        6310        6320        6330
TCA TTA GTT CAT AGC CCA TAT ATG GAG TTC CGC GTT ACA TAA CTT ACG
AGT AAT CAA GTA TCG GGT ATA TAC CTC AAG GCG CAA TGT ATT GAA TGC

6340        6350        6360        6370        6380
GTA AAT GGC CCG CCT GGC TGA CCG CCC AAC GAC CCC CGC CCA TTG ACG
CAT TTA CCG GGC GGA CCG ACT GGC GGG TTG CTG GGG GCG GGT AAC TGC

6390        6400        6410        6420        6430
TCA ATA ATG ACG TAT GTT CCC ATA GTA ACG CCA ATA GGG ACT TTC CAT
AGT TAT TAC TGC ATA CAA GGG TAT CAT TGC GGT TAT CCC TGA AAG GTA

6440        6450        6460        6470        6480
TGA CGT CAA TGG GTG GAG TAT TTA CGG TAA ACT GCC CAC TTG GCA GTA
ACT GCA GTT ACC CAC CTC ATA AAT GCC ATT TGA CGG GTG AAC CGT CAT

6490        6500        6510        6520        6530
CAT CAA GTG TAT CAT ATG CCA AGT ACG CCC CCT ATT GAC GTC AAT GAC
GTA GTT CAC ATA GTA TAC GGT TCA TGC GGG GGA TAA CTG CAG TTA CTG

6540        6550        6560        6570
GGT AAA TGG CCC GCC TGG CAT TAT GCC CAG TAC ATG ACC TTA TGG GAC
CCA TTT ACC GGG CGG ACC GTA ATA CGG GTC ATG TAC TGG AAT ACC CTG

6580        6590        6600        6610        6620
TTT CCT ACT TGG CAG TAC ATC TAC GTA TTA GTC ATC GCT ATT ACC ATG
AAA GGA TGA ACC GTC ATG TAG ATG CAT AAT CAG TAG CGA TAA TGG TAC

6630        6640        6650        6660        6670
GTG ATG CGG TTT TGG CAG TAC ATC AAT GGG CGT GGA TAG CGG TTT GAC
CAC TAC GCC AAA ACC GTC ATG TAG TTA CCC GCA CCT ATC GCC AAA CTG
```

FIG. 5 M

```
  6680      6690      6700      6710      6720
TCA CGG GGA TTT CCA AGT CTC CAC CCC ATT GAC GTC AAT GGG AGT TTG
AGT GCC CCT AAA GGT TCA GAG GTG GGG TAA CTG CAG TTA CCC TCA AAC

  6730      6740      6750      6760      6770
TTT TGG CAC CAA AAT CAA CGG GAC TTT CCA AAA TGT CGT AAC AAC TCC
AAA ACC GTG GTT TTA GTT GCC CTG AAA GGT TTT ACA GCA TTG TTG AGG

  6780      6790      6800      6810
GCC CCA TTG ACG CAA ATG GGC GGT AGG CGT GTA CGG TGG GAG GTC TAT
CGG GGT AAC TGC GTT TAC CCG CCA TCC GCA CAT GCC ACC CTC CAG ATA

6820      6830      6840      6850      6860
ATA AGC AGA GCT CGT TTA GTG AAC CGT CAG ATC GCC TGG AGA CGC CAT
TAT TCG TCT CGA GCA AAT CAC TTG GCA GTC TAG CGG ACC TCT GCG GTA

6870      6880      6890      6900      6910
CCA CGC TGT TTT GAC CTC CAT AGA AGA CAC CGG GAC CGA TCC AGC CTC
GGT GCG ACA AAA CTG GAG GTA TCT TCT GTG GCC CTG GCT AGG TCG GAG

6920      6930      6940      6950      6960
CGC GGC CGG GAA CGG TGC ATT GGA ACG CGG ATT CCC CGT GCC AAG AGT
GCG CCG GCC CTT GCC ACG TAA CCT TGC GCC TAA GGG GCA CGG TTC TCA

  6970      6980      6990      7000      7010
GAC GTA AGT ACC GCC TAT AGA GTC TAT AGG CCC ACC CCC TTG GCT TCT
CTG CAT TCA TGG CGG ATA TCT CAG ATA TCC GGG TGG GGG AAC CGA AGA

  7020      7030      7040      7050
TAT GCA TGC TAT ACT GTT TTT GGC TTG GGG TCT ATA CAC CCC CGC TTC
ATA CGT ACG ATA TGA CAA AAA CCG AAC CCC AGA TAT GTG GGG GCG AAG

7060      7070      7080      7090      7100
CTC ATG TTA TAG GTG ATG GTA TAG CTT AGC CTA TAG GTG TGG GTT ATT
GAG TAC AAT ATC CAC TAC CAT ATC GAA TCG GAT ATC CAC ACC CAA TAA

7110      7120      7130      7140      7150
GAC CAT TAT TGA CCA CTC CCC TAT TGG TGA CGA TAC TTT CCA TTA CTA
CTG GTA ATA ACT GGT GAG GGG ATA ACC ACT GCT ATG AAA GGT AAT GAT

7160      7170      7180      7190      7200
ATC CAT AAC ATG GCT CTT TGC CAC AAC TCT CTT TAT TGG CTA TAT GCC
TAG GTA TTG TAC CGA GAA ACG GTG TTG AGA GAA ATA ACC GAT ATA CGG

  7210      7220      7230      7240      7250
AAT ACA CTG TCC TTC AGA GAC TGA CAC GGA CTC TGT ATT TTT ACA GGA
TTA TGT GAC AGG AAG TCT CTG ACT GTG CCT GAG ACA TAA AAA TGT CCT
```

FIG. 5 N

7260 7270 7280 7290

TGG GGT CTC ATT TAT TAT TTA CAA ATT CAC ATA TAC AAC ACC ACC GTC
ACC CCA GAG TAA ATA ATA AAT GTT TAA GTG TAT ATG TTG TGG TGG CAG

7300 7310 7320 7330 7340

CCC AGT GCC CGC AGT TTT TAT TAA ACA TAA CGT GGG ATC TCC ACG CGA
GGG TCA CGG GCG TCA AAA ATA ATT TGT ATT GCA CCC TAG AGG TGC GCT

7350 7360 7370 7380 7390

ATC TCG GGT ACG TGT TCC GGA CAT GGG CTC TTC TCC GGT AGC GGC GGA
TAG AGC CCA TGC ACA AGG CCT GTA CCC GAG AAG AGG CCA TCG CCG CCT

7400 7410 7420 7430 7440

GCT TCT ACA TCC GAG CCC TGC TCC CAT GCC TCC AGC GAC TCA TGG TCG
CGA AGA TGT AGG CTC GGG ACG AGG GTA CGG AGG TCG CTG AGT ACC AGC

7450 7460 7470 7480 7490

CTC GGC AGC TCC TTG CTC CTA ACA GTG GAG GCC AGA CTT AGG CAC AGC
GAG CCG TCG AGG AAC GAG GAT TGT CAC CTC CGG TCT GAA TCC GTG TCG

7500 7510 7520 7530

ACG ATG CCC ACC ACC ACC AGT GTG CCG CAC AAG GCC GTG GCG GTA GGG
TGC TAC GGG TGG TGG TGG TCA CAC GGC GTG TTC CGG CAC CGC CAT CCC

7540 7550 7560 7570 7580

TAT GTG TCT GAA AAT GAG CTC GGG GAG CGG GCT TGC ACC GCT GAC GCA
ATA CAC AGA CTT TTA CTC GAG CCC CTC GCC CGA ACG TGG CGA CTG CGT

7590 7600 7610 7620 7630

TTT GGA AGA CTT AAG GCA GCG GCA GAA GAA GAT GCA GGC AGC TGA GTT
AAA CCT TCT GAA TTC CGT CGC CGT CTT CTT CTA CGT CCG TCG ACT CAA

7640 7650 7660 7670 7680

GTT GTG TTC TGA TAA GAG TCA GAG GTA ACT CCC GTT GCG GTG CTG TTA
CAA CAC AAG ACT ATT CTC AGT CTC CAT TGA GGG CAA CGC CAC GAC AAT

7690 7700 7710 7720 7730

ACG GTG GAG GGC AGT GTA GTC TGA GCA GTA CTC GTT GCT GCC GCG CGC
TGC CAC CTC CCG TCA CAT CAG ACT CGT CAT GAG CAA CGA CGG CGC GCG

7740 7750 7760 7770

GCC ACC AGA CAT AAT AGC TGA CAG ACT AAC AGA CTG TTC CTT TCC ATG
CGG TGG TCT GTA TTA TCG ACT GTC TGA TTG TCT GAC AAG GAA AGG TAC

7780 7790 7800 7810 7820

GGT CTT TTC TGC AGT CAC CGT CCT TGA CAC GAA GCT TGG GCT GCA GGT
CCA GAA AAG ACG TCA GTG GCA GGA ACT GTG CTT CGA ACC CGA CGT CCA

FIG. 5 O

```
7830                7840                7850                7860
  *                   *                   *                   *
CGA TCG ACT CTA GAG GAT CGA TCC CCG GGC GAG CTC G
GCT AGC TGA GAT CTC CTA GCT AGG GGC CCG CTC GAG C
```

FIG. 6

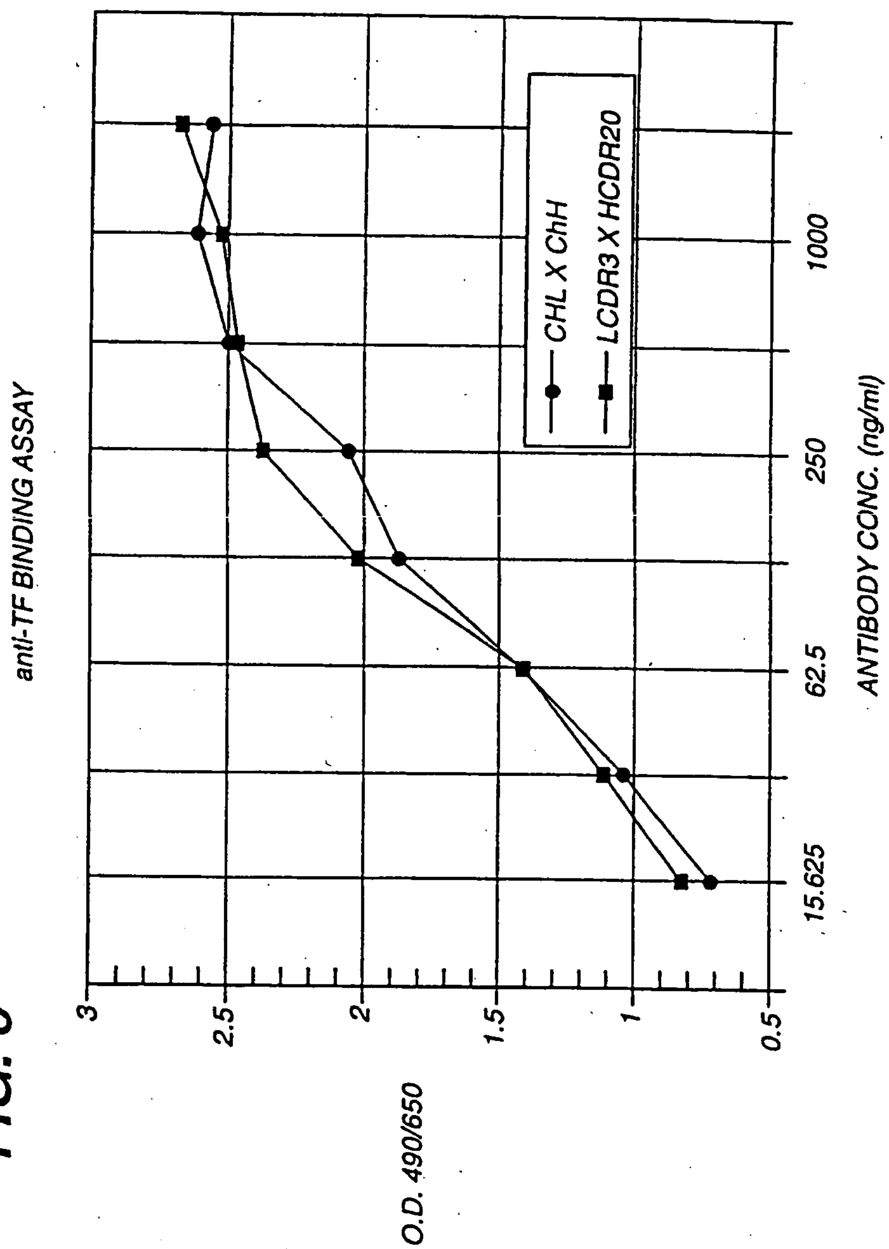

anti-TF BINDING ASSAY
CHL X ChH
LCDR3 X HCDR20
O.D. 490/650
3
2.5
2
1.5
1
0.5
15.625
62.5
250
1000
ANTIBODY CONC. (ng/ml)

FIG. 7

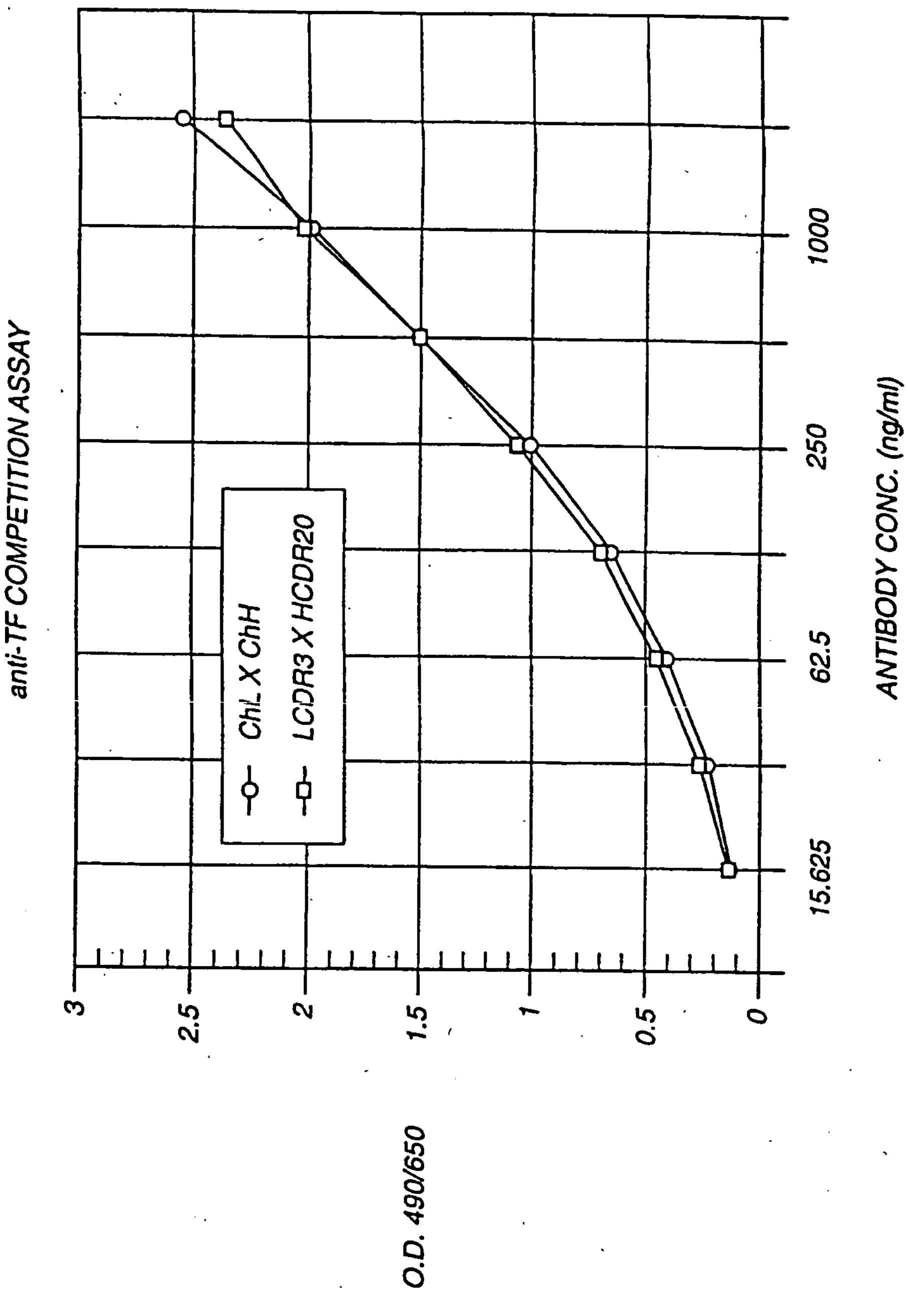

anti-TF COMPETITION ASSAY
ChL X ChH
LCDR3 X HCDR20
O.D. 490/650
3
2.5
2
1.5
1
0.5
0
15.625
62.5
250
1000
ANTIBODY CONC. (ng/ml)

FIG. 8

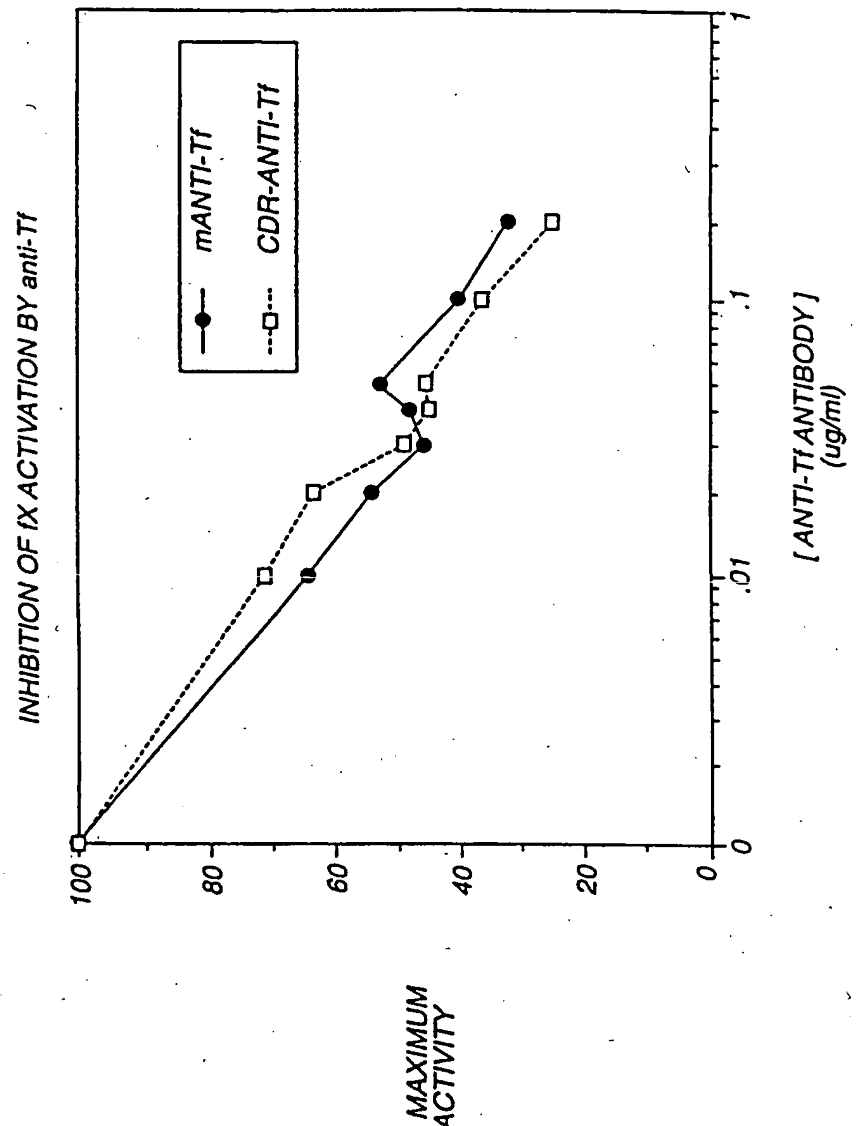

INHIBITION OF fX ACTIVATION BY anti-Tf
mANTI-Tf
CDR-ANTI-Tf
% MAXIMUM ACTIVITY
100
80
60
40
20
0
0
.01
.1
1
[ANTI-Tf ANTIBODY] (ug/ml)

FIG. 9

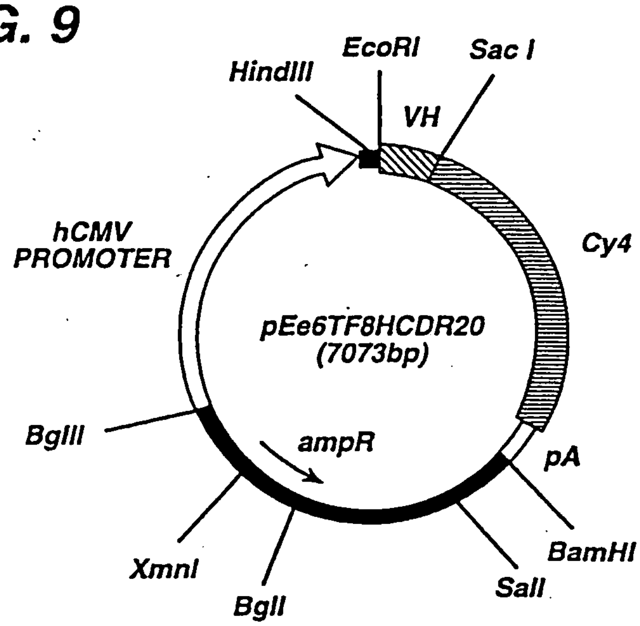
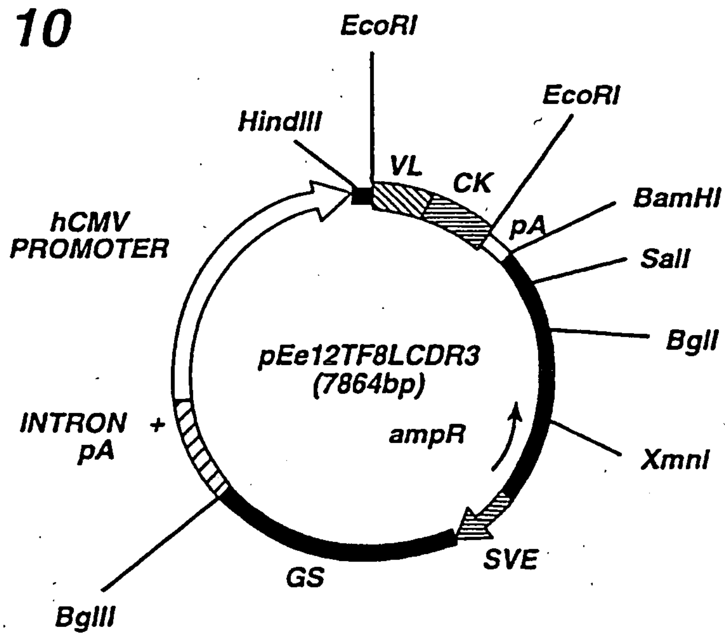

EcoRI
Sac I
HindIII
VH
hCMV
PROMOTER
Cγ4
pEe6TF8HCDR20
(7073bp)
BglII
ampR
pA
BamHI
XmnI
BglI
SalI

FIG. 10


EcoRI
EcoRI
HindIII
VL
CK
BamHI
hCMV
PROMOTER
pA
SalI
BglI
pEe12TF8LCDR3
(7864bp)
INTRON +
pA
ampR
XmnI
SVE
GS
BglII

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/09287

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/13 C07K16/36 C07K16/46 A61K39/395 //C12N5/10, C12N15/85

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 91 09968 A (CELLTECH LIMITED) 11 July 1991 see examples see claims	1-37
Y	WO 88 07543 A (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 6 October 1988 see claims	1-37
A	WO 94 11029 A (THE SCRIPPS RESEARCH INSTITUTE ET AL.) 26 May 1994 see claims	1-37
A	WO 94 05328 A (THE SCRIPPS RESEARCH INSTITUTE) 17 March 1994 see examples see claims	1-37

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
15 October 1996	08.11.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/09287

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF CRYSTAL GROWTH, vol. 122, no. 1-4, August 1992, AMSTERDAM, NL, pages 253-264, XP002015918 W. RUF ET AL.: "Purification, sequence and crystallization of an anti-tissue factor Fab and its use for the crystallization of tissue factor." see abstract see table 1	1-37

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 96/09287

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 31-35
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 31-35 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/09287

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9109968	11-07-91	AT-T- 129017	15-10-95
		AT-T- 124459	15-07-95
		AU-B- 664801	30-11-95
		AU-A- 6461294	22-12-94
		AU-B- 646009	03-02-94
		AU-A- 6974091	24-07-91
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